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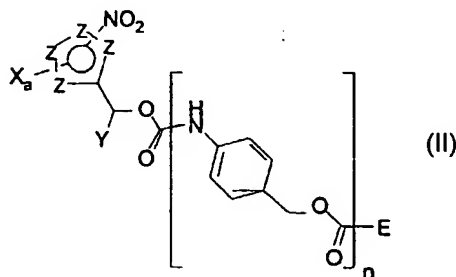
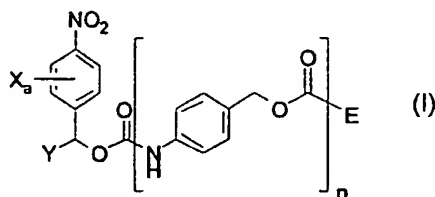
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(54) Title: N-PROTECTED AMINES AND THEIR USE AS PRODRUGS



(57) Abstract: Compounds of formula (I) or (II), wherein X represents H, C₁₋₆ alkyl or C₁₋₆ alkoxy, said alkyl or alkoxy being optionally substituted with one or more groups; a is 0,1,2,3 or 4; Y represents H or C₁₋₆ alkyl; 1, 2 or 3 of the members Z of the 5-membered aromatic ring are independently selected from -O-, -S-, -N= or -NR-, where R is H or C₁₋₆ alkyl optionally substituted with one or more of groups; and E represents a moiety such that EH is an amine; provided that in formula (I) if a = 0 then Y ≠ H, are provided along with a method of selecting desired protecting groups by measuring the fragmentation rates of compounds of formula (I) or (II) when the nitro group is selected.



For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

N-PROTECTED AMINES AND THEIR USE AS PRODRUGS

The present invention relates to methods and compounds for providing amines with N-protecting groups. It further relates to the protected amines themselves and their use as
5 prodrugs.

The amines are protected as nitroaromatic carbamates (where "aromatic" includes "heteroaromatic"). They include nitro groups which are susceptible to reduction, leading to loss of the protecting group and liberation of the amine. The amine is desirably biologically active, the activity being substantially suppressed by N-protection. Thus it
10 may be an amine-based cytotoxin, e.g actinomycin D, an anthracycline, an aniline mustard or an enediyne.

Thus suitable protected amines may be valuable as anticancer agents, and/or may be useful as prodrugs for antibody-directed enzyme prodrug therapy (ADEPT) or gene-directed enzyme prodrug therapy (GDEPT), in conjunction with nitroreductase enzymes.

15 BACKGROUND TO THE INVENTION

The use of prodrugs (relatively inactive compounds which can be converted into more active compounds in vivo) represents a clinically very valuable concept, particularly in cancer therapy. For example a prodrug may be converted into an anti-tumour agent under the influence of an enzyme that is linkable to a monoclonal antibody that will bind to a tumour
20 associated antigen. The combination of such a prodrug with such an enzyme monoclonal/antibody conjugate represents a very powerful clinical agent. This approach to cancer therapy, often referred to as "antibody directed enzyme/prodrug therapy" (ADEPT), is disclosed in WO88/07378.

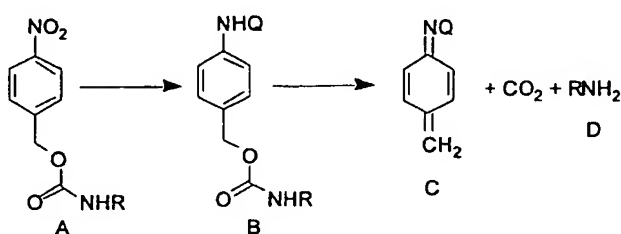
A further therapeutic approach termed "virus-directed enzyme prodrug therapy" (VDEPT) has been proposed as a method for treating tumour cells in patients using prodrugs. Tumour cells are targeted with a viral vector carrying a gene encoding an enzyme capable of activating a prodrug. The gene may be transcriptionally regulated by tissue specific promoter or enhancer sequences. The viral vector enters tumour cells and expresses the enzyme, in order that a prodrug is converted to an active drug within the tumour cells (*Huber et al.*, Proc.
30 Natl. Acad. Sci. USA (1991) 88, 8039). Alternatively, non-viral methods for the delivery of genes have been used. Such methods include calcium phosphate co-precipitation, microinjection, liposomes, direct DNA uptake, and receptor-mediated DNA transfer. These

- 2 -

are reviewed in Morgan & French, *Annu. Rev. Biochem.*, 1993, 62;191. The term "GDEPT" (gene-directed enzyme prodrug therapy) is used to include both viral and non-viral delivery systems.

4-Nitrobenzyl carbamates (A) undergo multi-electron reduction to produce amines.

- 5 The mechanism probably involves the formation of electron-donating 4-hydroxylamine (B; Q=OH) or 4-amine (B; Q=H) species, which then fragment to generate a quinoneimine methide (C) and an amine (D) [P.L. Carl, P.K. Charkravarty, and J.A. Katzenellenbogen, *J. Med. Chem.*, 1981, **24**, 479].

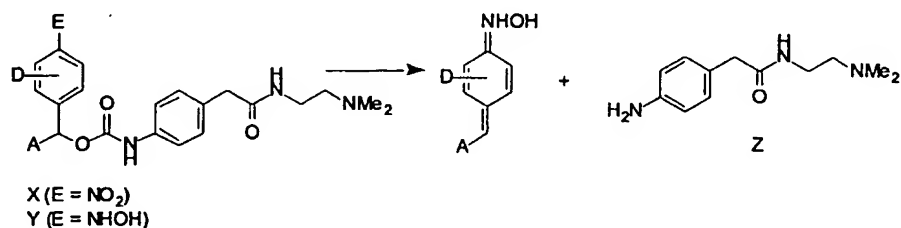


- Despite a low reduction potential (ca. -490 mV) [P. Wardman, *Environ. Health Perspect.*, 1985, **64**, 309] the 4-nitrobenzyl carbamate moiety undergoes facile reduction by the *E. coli* NR enzyme, and has been used as a prodrug "trigger" to deactivate highly cytotoxic amine "effectors" [M.P. Hay and W.A. Denny, *Drugs Future*, 1996, **21**, 917]. The *E. coli* enzyme has been shown to activate 4-nitrobenzyl carbamate derivatives of a limited number of amine-based cytotoxins, including actinomycin D and anthracyclines [A.B. Mauger, P.J. Burke, H.H. Somani, F. Friedlos and R.J. Knox, *J. Med. Chem.*, 1994, **37**, 3452], aniline mustards [A.B. Mauger, P.J. Burke, H.H. Somani, F. Friedlos and R.J. Knox, *J. Med. Chem.*, 1994, **37**, 3452; M. Lee, J.E. Simpson Jr, S. Woo, C. Kaenzig, G.M. Anlezark, E. Eno-Amooquaye, and P.J. Burke, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 1065] and enediynes [M.P. Hay, W.R. Wilson, and W.A. Denny, *Bioorg. Med. Chem. Lett.*, 1995, **5**, 2829]. All of these studies have used the otherwise unsubstituted 4-nitrobenzyl carbamate moiety.
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- To be fully effective, such prodrugs must be activated efficiently by the enzyme, and the resulting reduced species must fragment rapidly to release the cytotoxic amine effector. Kinetic structure-activity relationships (SAR) have been extensively studied for the one-electron reduction of nitrobenzyl halides [D.L. Kirkpatrick, K.E. Johnson, and A.C. Sartorelli, *J. Med. Chem.*, 1986, **29**, 2048] and quaternary salts [M. Tercel, W.R. Wilson,
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R.F. Anderson, and W.A. Denny, *J. Med. Chem.*, 1996, 39, 1084 and refs therein], but not for 4-nitrobenzyl carbamates. We have found that suitable substituents on the 4-nitrobenzyl ring and/or alpha-carbon result in more rapid fragmentation of the 4-hydroxylamine intermediates, and can also serve as sites for attaching solubilising functionalities.

- 5 For a series of substituted 4-nitrobenzyl carbamate model compounds (X), fragmentation rates of the corresponding 4-hydroxylamines (Y) to release amines (Z) correlated with electron-donating properties (σ_p) of the substituent, as shown in Table 1. The maximum half-lives ($Mt_{1/2}$) of the hydroxylamine derivatives were measured by HPLC, following 4-fold stoichiometry radiolytic reduction of the corresponding substituted 4-
- 10 nitrobenzyl carbamates. Assuming first order conditions, the half-life ($t_{1/2}$) of species R is calculated from the equation $\ln([R]_0/[R]_t) = t(\ln 2/t_{1/2})$. The ratio $[R]_0/[R]_t$ was taken as the fraction of nitrobenzyl carbamate which had not released the amine (Z) after 4-fold reduction. This method yields a maximum value for the half-life of fragmentation.



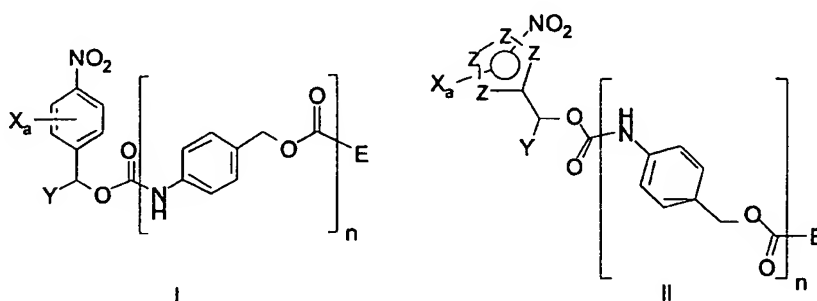
- Table 1 Half-lives for fragmentation ($Mt_{1/2}$) and percent of amine released (t_0) for
- 15 substituted 4-hydroxylaminobenzyl carbamates (derived from the corresponding 4-nitrobenzyl carbamates by radiolytic reduction).

D	A	σ_p	$Mt_{1/2}$ (min)	t_0 (%)
2-NO ₂	H	0.78	88	18
3-NO ₂	H	0.71	65	22
3-CO ₂ Me	H	0.37	20	44
3-OMe	H	0.12	17	37
H	H	0.0	16	40
2-OMe	H	-0.27	12	48
2-NHMe	H	-0.84	7	65
H	Me	0.0	9.5	-

Table 1 shows that the unsubstituted hydroxylaminobenzyl carbamate normally used as a trigger has a half-life of 16 minutes. This is relatively long and, under biological conditions, may result in substantial loss of material by side reactions not involving (activating) amine release. The half-life can be lowered significantly by the use of electron-donating substituents, and/or by the use of α -substituents (A).

DISCLOSURE OF THE INVENTION

In a first aspect, the invention provides a method of providing an amine with a protecting group comprising (i) providing a plurality of different compounds selected from compounds of formulae (I) and (II)



wherein:

X represents H, C₁₋₆ alkyl or C₁₋₆ alkoxy, said alkyl or alkoxy being optionally substituted with one or more of the following groups: hydroxy (OH), ether (OR_x), amino (NH₂), mono-substituted amino (NR_xH), di-substituted amino (NR_x¹R_x²), cyclic C₁₋₅ alkylamino, imidazolyl, C₁₋₆ alkylpiperazinyl, morpholino, thiol (SH), thioether (SR_x), tetrazole, carboxy (COOH), carboxylate (COOR_x), sulphony (S(=O)₂OH), sulphonate

- 5 -

- (S(=O)₂OR_x), sulphonyl (S(=O)₂R_x), sulphixy (S(=O)OH), sulphinate (S(=O)OR_x), sulphinyl (S(=O)R_x), phosphonooxy (OP(=O)(OH)₂) and phosphate (OP(=O)(OR_x)₂), where R_x, R_x¹ and R_x² are selected from a C₁₋₆ alkyl group, a C₃₋₂₀ heterocyclyl group or a C₅₋₂₀ aryl group, preferably a C₁₋₆ alkyl group; a is 0, 1, 2, 3 or 4; Y represents H or C₁₋₆ alkyl; 1, 2 or 3 of the
- 5 members Z of the 5-membered aromatic ring are independently selected from -O-, -S-, -N= or -NR-, (where R is H or C₁₋₆ alkyl optionally substituted with one or more of the following groups: hydroxy (OH), ether (OR_R), amino (NH₂), mono-substituted amino (NR_RH), di-substituted amino (NR_R¹R_R²), C₁₋₅ cyclic amino, imidazolyl, alkylpiperazinyl, morpholino, thiol (SH), thioether (SR_R), tetrazole, carboxy (COOH), carboxylate (COOR_R), sulphonyl (S(=O)₂OH), sulphonate (S(=O)₂OR_R), sulphonyl (S(=O)₂R_R), sulphixy (S(=O)OH), sulphinate (S(=O)OR_R), sulphinyl (S(=O)R_R), phosphonooxy (OP(=O)(OH)₂) and phosphate (OP(=O)(OR_R)₂), where R_R, R_R¹ and R_R² are selected from a C₁₋₆ alkyl group, a C₃₋₂₀ heterocyclyl group or a C₅₋₂₀ aryl group, preferably a C₁₋₆ alkyl group), the other ring atoms being C; n is 0 or 1; and E represents a moiety such that EH is an amine;
- 10 (ii) measuring the rates of fragmentation of the compounds to release EH when the nitro group is reduced and selecting a compound having a desired rate of decomposition; and
- (iii) providing the amine to be protected with a protecting group corresponding to that in the selected compound.

In this aspect, the step of selecting the compound is preferably carried out in order to

20 provide a protecting group with a faster rate of fragmentation than unsubstituted 4-nitrobenzyl carbamate. However selecting a compound bearing a protecting group with a slower rate of fragmentation than 4-nitrobenzyl carbamate may be preferred. This particularly applies in situations in which it is desired to provide a prodrug which can diffuse away from the site of actuation by the appropriate enzyme, and thus kill tumour cells further away from the site of

25 actuation (the "bystander" effect).

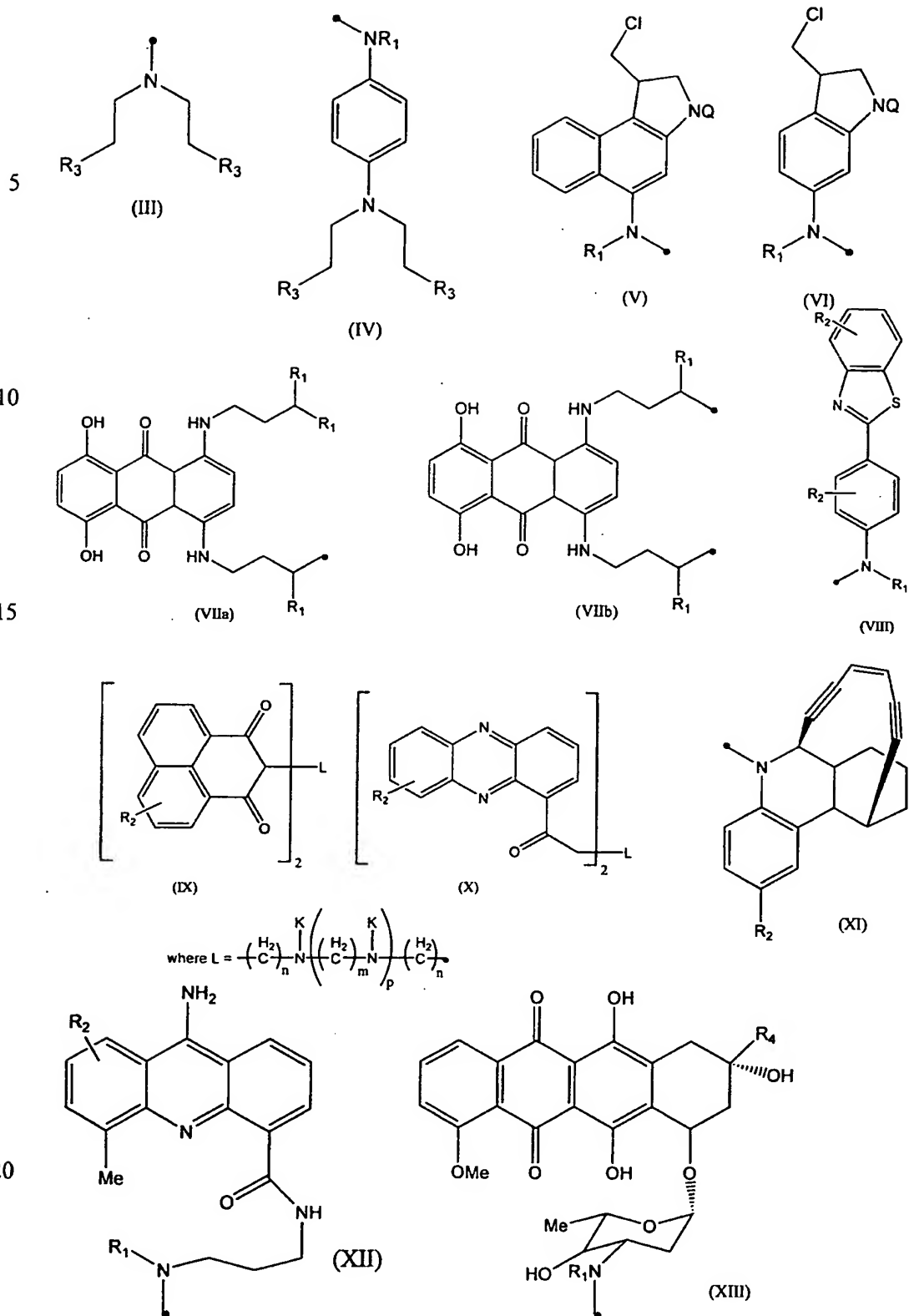
In a second aspect, the present invention relates to a compound represented by the general formula (I) or (II) as shown above, wherein X, Y, Z, E, a and n are as defined above; provided that in formula (I) if a = 0 then Y ≠ H.

EH is preferably a cytotoxic amine. E may be selected from formulae (III-XIII).

- 30 In (III-XIII), R₁ represents H or C₁₋₆ alkyl, being optionally substituted with one or more of the following groups: one or more of the following groups: hydroxy (OH), ether (OR_E), amino (NH₂), mono-substituted amino (NR_EH), di-substituted amino (NR_E¹R_E²), cyclic

- 6 -

- C_{1-5} alkylamino, imidazolyl, C_{1-6} alkylpiperazinyl, morpholino, thiol (SH), thioether (SR_E), tetrazole, carboxy (COOH), carboxylate ($COOR_E$), sulphonyl ($S(=O)_2OH$), sulphinate ($S(=O)_2OR_E$), sulphonyl ($S(=O)_2R_E$), sulphoxy ($S(=O)OH$), sulphinate ($S(=O)OR_E$), sulphiny ($S(=O)R_E$), phosphonoxy ($OP(=O)(OH)_2$) and phosphate ($OP(=O)(OR_E)_2$), where R_E , R_E^1 and R_E^2 are selected from a C_{1-6} alkyl group, a C_{3-20} heterocyclyl group or a C_{5-20} aryl group, more preferably from a C_{1-6} alkyl group; R_2 represents H, C_{1-6} alkyl, C_{1-6} alkoxy, OH, halogen, NO_2 , NH_2 , $NHMe$, NMe_2 , SO_2Me , CF_3 , CN, $CONH_2$ or $CONHMe$; each R_3 is independently selected from Cl, Br, I and OMS; and R_4 is selected from $-C(=O)Me$ and $-C(=O)CH_2OH$; Q represents substituted indole, substituted benzofuran or substituted cinnamoyl; in (IX) and (X), each n is independently from 2-4, and each m is independently from 2-4, and p = 0 or 1.



Compounds of formula V are described in EP 0 938 474, which is incorporated herein by reference. Compounds of formula VI are described in EP 0 850 220, which is incorporated herein by reference.

A compound of formula (I) or (II) may be basic or acidic and may thus form
5 pharmaceutically acceptable salts with both organic and inorganic acids and bases. These are included within the scope of the second aspect.

In a first type of preferred embodiment, X represents C_{1-6} alkyl or C_{1-6} alkoxy, said alkyl or alkoxy being optionally substituted with one or more of the following groups: hydroxy, ether (OR_x), amino, alkylamino (NR_xH), dialkylamino ($NR_x^1R_x^2$), cyclic C_{1-5}
10 alkylamino, imidazolyl, C_{1-6} alkylpiperazinyl, morpholino, thiol, alkylthioether (SR_x), tetrazole and $-CO_2X'$ where X' is selected from the possibilities listed for X and R_x , R_x^1 and R_x^2 are selected from C_{1-6} alkyl; a is 0,1,2,3 or 4; Y represents H or lower alkyl; 1, 2 or 3 of the members Z of the 5-membered aromatic ring are independently selected from -O-, -S-, -N= or -NR-, (where R is H or lower alkyl optionally substituted with one or more of the following
15 groups: hydroxy, ether (OR_R), amino, alkylamino (NR_RH), dialkylamino ($NR_R^1R_R^2$), cyclic C_{1-5} alkylamino, imidazolyl, C_{1-6} alkylpiperazinyl, morpholino, thiol, alkylthioether (SR_R), tetrazole and $-CO_2R'$ where R' is selected from the possibilities listed for R and R_R , R_R^1 and R_R^2 are selected from C_{1-6} alkyl); and E represents R_1 represents H or C_{1-6} alkyl, being optionally substituted with one or more of the following groups hydroxy, ether (OR_E), amino, alkylamino
20 (NR_EH), dialkylamino ($NR_E^1R_E^2$), cyclic C_{1-5} alkylamino, imidazolyl, C_{1-6} alkylpiperazinyl, morpholino, thiol, alkylthioether (SR_E), tetrazole and $-CO_2X'$ where X' is selected from the possibilities listed for X and R_E , R_E^1 and R_E^2 are selected from C_{1-6} alkyl.

In a second type of preferred embodiment, the compound is of formula (I) where a is
1. It is further preferred in this embodiment that X represents optionally substituted C_{1-6}
25 alkoxy and more preferably in the 2 position. The alkoxy group is preferably selected from methyl, ethyl and n-propyl, and the substituents from hydroxy, methoxy, phosphonoxy, NMe_2 , $Nmorph$, OCO_2tBu , and OCO_2H . In more preferred embodiments the ethyl or n-propyl group is singly substituted, most preferably with hydroxy, whereas the Me group is unsubstituted.

In this type of embodiment Y is preferably selected from H or Me. E is preferably
30 selected from III - XIII, most preferably V or XIII. If E is selected from XIII, n is preferably 1.

In a third preferred type of embodiment, the compound is of formula (II) with one Z

being -N= and another Z being -NR-, R being preferably Me or Et, the other members of the heterocyclic ring being C. If R is Et, it is preferably substituted with hydroxy. A is preferably 0. More preferably the -N= and -NR- are not adjacent in the heterocyclic ring. The most preferred arrangement is 2 (-NMe-) and 5 (-N=), with the NO₂ at the 3 position. In this type of embodiment, Y is preferably selected from H or Me. E is preferably selected from V or XIII. If E is selected from XIII, than n is preferably 1.

In a fourth preferred type of embodiment, the compound is of formula (II), a is 0, and Z is either O or S. It is further preferred that the O or S is in the 2 position in the ring, and the NO₂ is attached to the 3 position. In this type of embodiment, Y is preferably selected from H or Me.

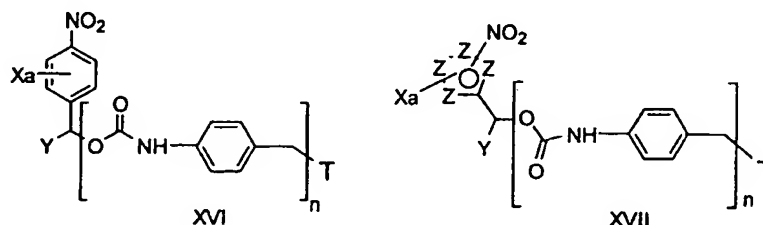
In a fifth preferred embodiment, the compound is of formula (II), and Z is NR, preferably NMe. There may be one further substituent (a=1), or there may be no further substituents on the ring (a=0). The further substituent is preferably CO₂Et. In this type of embodiment, Y is preferably selected from H or Me.

In a third aspect the invention provides a compound according to the second aspect for pharmaceutical use.

In a fourth aspect the invention provides the use of a compound according to the second aspect for the manufacture of a composition for use in the treatment of a hyper-proliferative disease, particularly a neoplastic disease. The composition may also include activating means for simultaneous or separate administration, the activating means typically comprising an enzyme or means for providing an enzyme, for performing ADEPT or VDEPT therapy. The activating means typically leads to liberation of the amine EH.

In a fifth aspect the invention provides a compound of the formula XVI or XVII where X, n, Z and Y are as defined for the second aspect and T is OH or an activated alcohol functionality (such as -O.CO.L where L is a leaving group such as Cl) suitable for reaction with an amine EH to produce a compound according to the second aspect.

In a sixth aspect the invention provides the use of a compound of formula (XVI) or

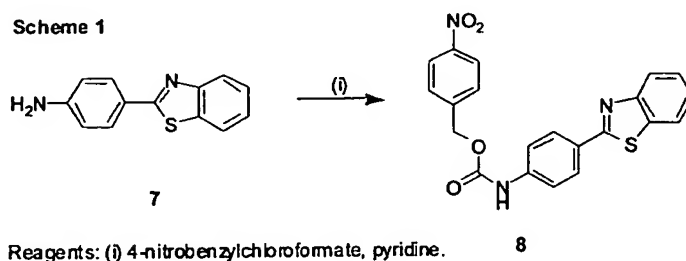


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(XVII) in protecting an amine. This may include activation of an alcohol (XVI or XVII where T is OH) with a reagent such as phosgene, diphosgene or triphosgene or a chloroformate, e.g. 4-nitrophenylchloroformate or pentafluorophenylchloroformate, optionally
 5 in conjunction with HOBT(1-hydroxybenzotriazole).

In a further aspect, the present invention relates to a method of preparing compounds of the general formula (I); examples of the methods are outlined in Schemes 1-24.

Thus (Scheme 1), reaction of the amine 7 [D-F. Shi, T. D. Bradshaw, S. Wrigley, C. J. McCall, P. Lelieveld, I. Fitchner, M. F. G. Stevens. *J. Med. Chem.*, 1996, 39, 3375] with 4-
 10 nitrobenzylchloroformate gave carbamate 8.

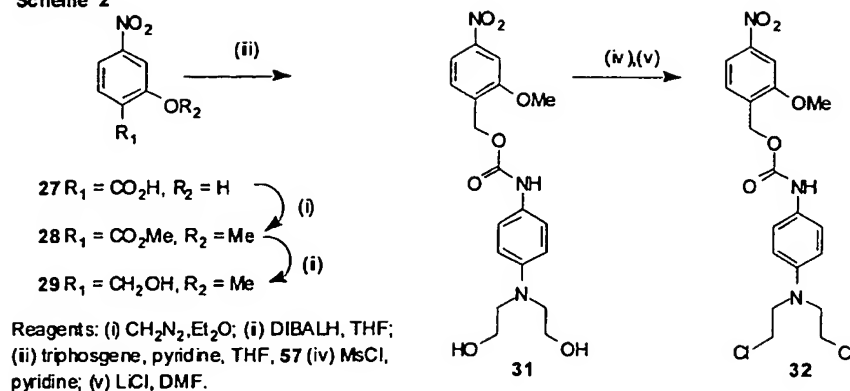


Reaction of the 1,4-difluoro-5,8-dihydroxyanthracene-9,10-dione 22 with amine 15 in pyridine gave the monocarbamate 23 and biscarbamate 24 (Scheme 7). Further reaction of 23 with 2-(2-aminoethylamino)ethanol gave carbamate 26.

15 In another example (Scheme 2), 4-nitrosalicylic acid (27) was methylated using a solution of diazomethane in ether and the methyl ester 28 reduced with DIBALH in THF to give the nitrobenzyl alcohol 29. Activation of the alcohol 29 with triphosgene (or alternatively phosgene or diphosgene) in the presence of pyridine, and reaction with *N*¹,*N*¹-bis(2-hydroxyethyl)-1,4-benzenediamine (57) gives the carbamate 31, which was elaborated to the
 20 mustard 32 using standard methods.

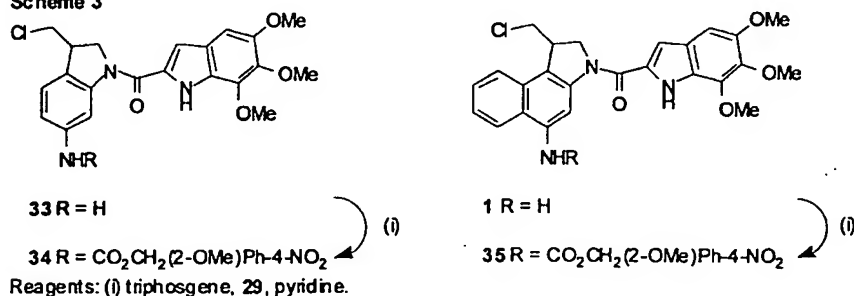
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Scheme 2



Similarly (Scheme 3), activation of alcohol 29 with triphosgene in the presence of pyridine and reaction with amine 33 [M. Tercel and W. A. Denny, *J. Chem. Soc. Perkin Trans. 1*, 1998, 509] or amine 1 [G. J. Atwell, W. R. Wilson, W. A. Denny, *Bioorg. Med. Chem. Lett.*, 1997, 7, 1483] gave carbamates 34 and 35, respectively.

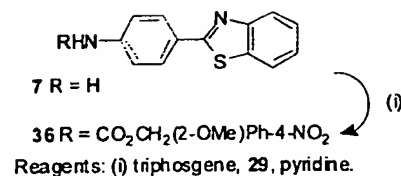
Scheme 3



Similarly (Scheme 4), activation of alcohol 29 with triphosgene in the presence of pyridine and reaction with amine 7 [D-F. Shi, T. D. Bradshaw, S. Wrigley, C. J. McCall, P. Lelieveld, I. Fitchner, M. F. G. Stevens, *J. Med. Chem.*, 1996, 39, 3375]] gave carbamate 36.

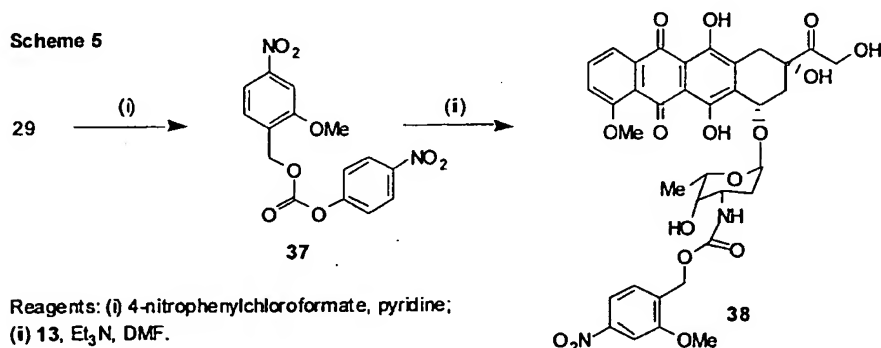
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Scheme 4



In another example (Scheme 5), activation of alcohol 29 with 4-nitrophenylchloroformate gave the carbonate 37 which was reacted with doxorubicin (13) and triethylamine in DMF to give the doxorubicin carbamate 38.

Scheme 5

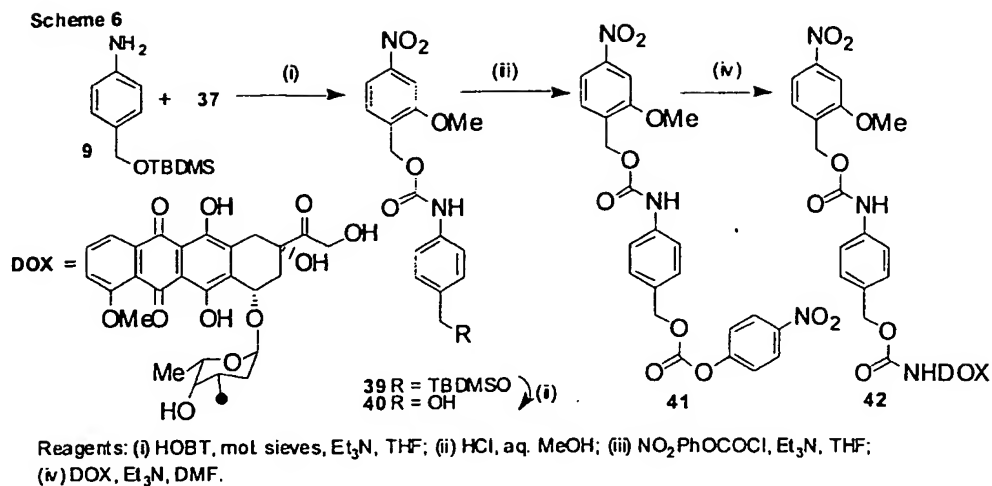


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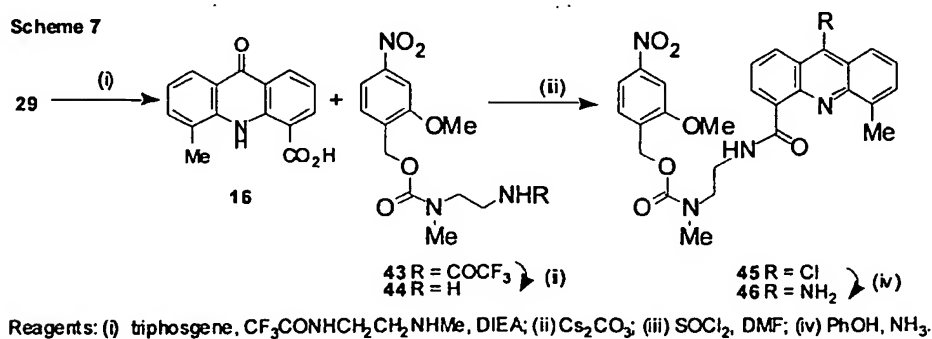
In another example (Scheme 6), carbonate 37 was coupled to amine 9 using 1-hydroxybenzotriazole (HOBt), 4Å molecular sieves and triethylamine to give protected carbamate 39. Removal of the TBDMS protecting group with aqueous acid gave the alcohol 40 which was activated with 4-nitrophenylchloroformate to give the carbonate 41. Reaction of 41 with doxorubicin (13) and triethylamine in DMF gave the carbamate 42.

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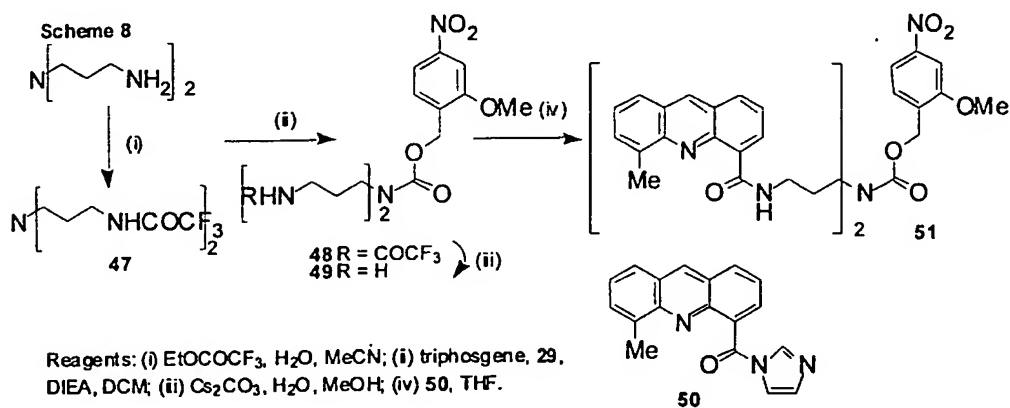
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In another example (Scheme 7), reaction of alcohol **29** with triphosgene and triethylamine, and coupling to 2,2,2-trifluoro-*N*-[2-(methylamino)ethyl]acetamide trifluoroacetate gave the trifluoroacetamide **43** which was deprotected under basic conditions to give amine **44**. Activation of the 5-methyl-9-oxo-9,10-dihydro-4-acridinecarboxylic acid (**16**) with thionyl chloride and coupling of the intermediate 9-chloroacridinyl acid chloride with amine **44** gave amide **45** which was converted to carbamate **46** using ammonia in phenol.



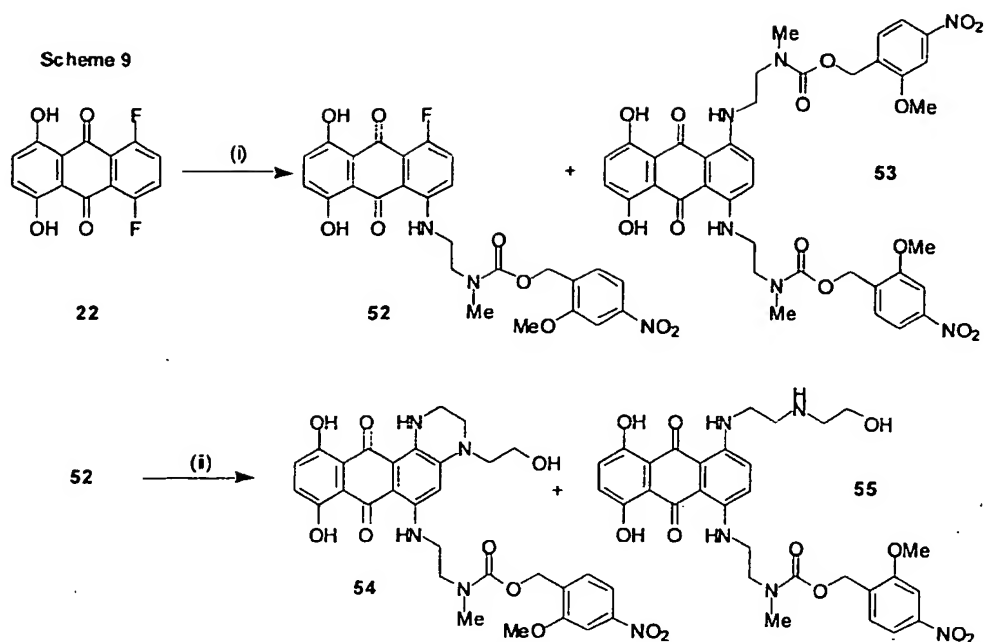
In another example (Scheme 8), the bistrifluoroacetamide **47** was coupled to alcohol **29** to give bisamide **48** which was deprotected under basic conditions to give the amine **49**. The amine **49** was coupled to 4-(1*H*-imidazol-1-ylcarbonyl)-5-methylacridine (**50**) [S. A. Gamage, J. A. Spicer, G. J. Atwell, G. J. Finlay, B. C. Baguley, W. A. Denny, *J. Med. Chem.*, 1999, 42, 2383-2393] to give the carbamate **51**.



Reaction of the 1,4-difluoro-5,8-dihydroxyanthracene-9,10-dione **22** with amine **44** gave the monocarbamate **52** and biscarbamate **53** (Scheme 9). Further reaction of **52** with 2-(2-aminoethylamino)ethanol gave carbamate **55**.

- 15 -

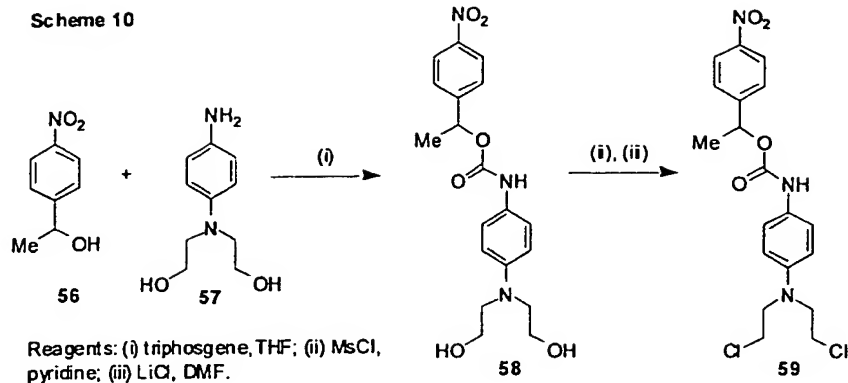
Scheme 9



Reagents: (i) 44, pyridine; (ii) $\text{NH}_2(\text{CH}_2)_2\text{NH}(\text{CH}_2)_2\text{OH}$, pyridine.

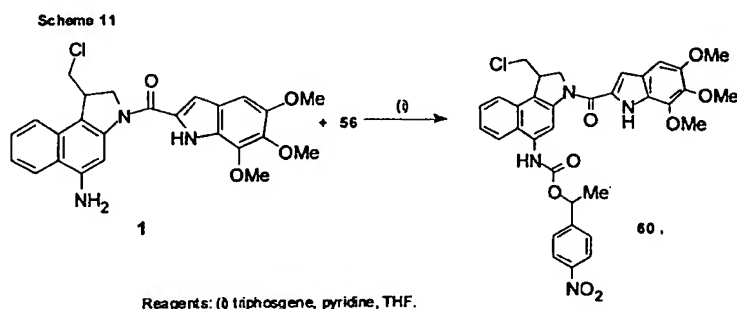
In another example (Scheme 10), reaction of 4-nitrophenylethan-1-ol (56) with triphosgene and pyridine, with the subsequent addition of N^1,N^1 -bis(2-hydroxyethyl)-1,4-benzenediamine (57) gave the carbamate 58 which was elaborated to the dichloride 59.

- 16 -



In another example (Scheme 11), coupling of the alcohol **56** with amine **1** [G. J. Atwell, W. R. Wilson, W. A. Denny, *Bioorg. Med. Chem. Lett.*, 1997, 7, 1483] using triphosgene and pyridine in THF gave the carbamate **60**.

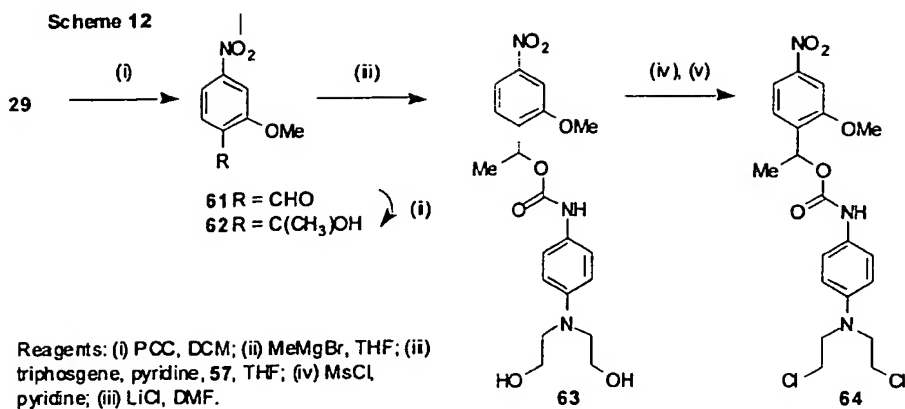
5



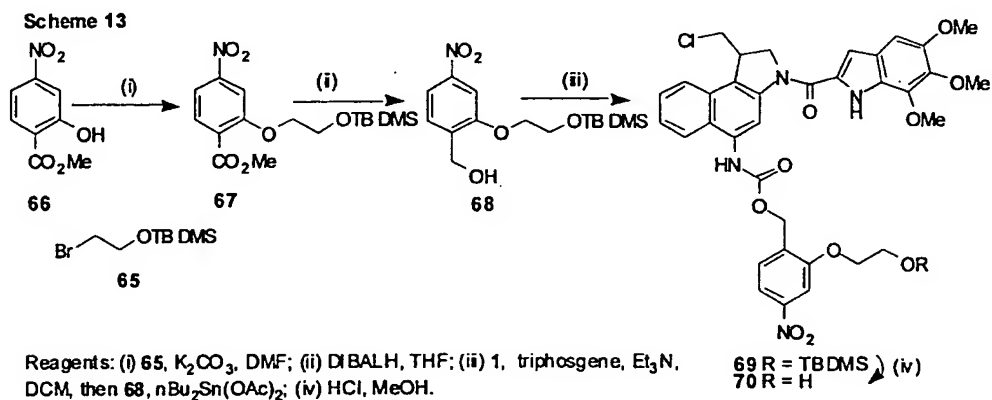
Oxidation of alcohol **29** with pyridinium chlorochromate (PCC) in DCM gave the aldehyde **61** (Scheme 12). Reaction of aldehyde **61** with methyl magnesium bromide in THF gave the alcohol **62** which was coupled to amine **57** to give carbamate diol **63**. The diol **63** was

10 elaborated to the dichloride **64** using standard methods.

- 17 -

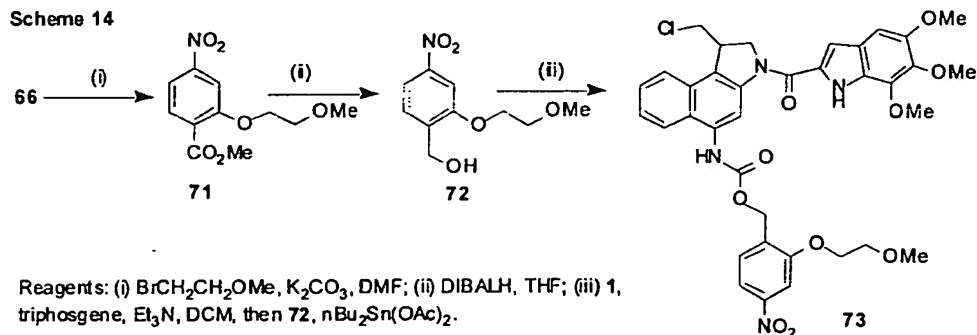


In another example (Scheme 13), alkylation of methyl 2-hydroxy-4-nitrobenzoate **66** with bromide **65** under basic conditions gave the ester **67** which was reduced to alcohol **68** using DIBALH in THF. Activation of amine **1** [G. J. Atwell, W. R. Wilson, W. A. Denny, *Bioorg. Med. Chem. Lett.*, 1997, 7, 1483] with triphosgene and triethylamine gave an intermediate isocyanate which was coupled with alcohol **68** using catalytic dibutyltin diacetate to give carbamate **69**. Deprotection of **69** under acidic conditions gave **70**.

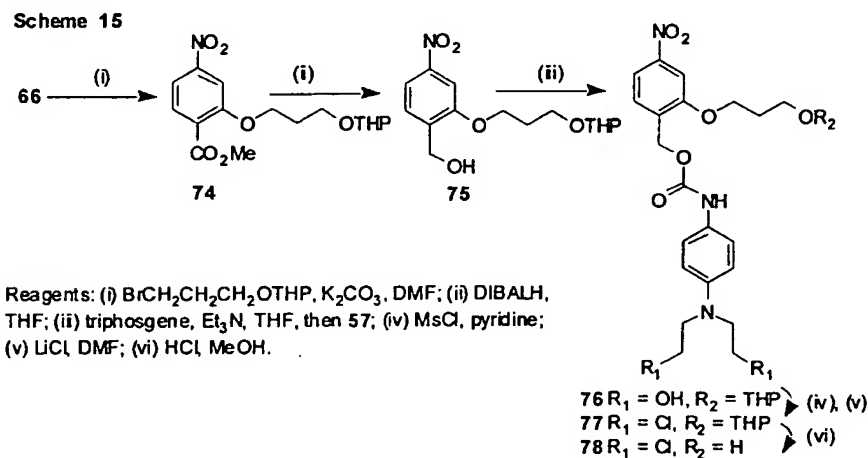


- 18 -

Similarly (Scheme 14), reaction of phenol **66** with 2-bromoethyl methyl ether gave ester **71** which was reduced to alcohol **72**. Activation of amine **1** [G. J. Atwell, W. R. Wilson, W. A. Denny, *Bioorg. Med. Chem. Lett.*, **1997**, *7*, 1483] with triphosgene and triethylamine gave an intermediate isocyanate which was coupled with alcohol **72** using catalytic dibutyltin diacetate to give carbamate **73**.



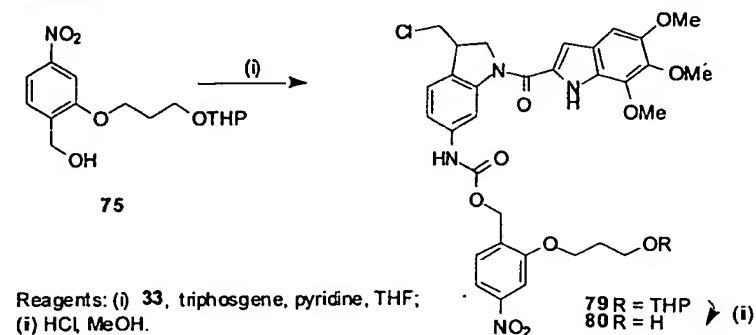
In another example (Scheme 15), phenol **66** was alkylated with 3-iodopropyl tetrahydropyranyl ether under basic conditions to give ester **74** which was reduced to alcohol **75** using DIBALH in THF. Activation of the alcohol **75** with triphosgene and triethylamine (or pyridine, or another organic base) in THF and subsequent reaction with amine **57** gave the carbamate diol **76**. The diol **76** was converted to the dichloride **77** using standard methods and the tetrahydropyranyl ether deprotected under acidic conditions to give carbamate **78**.



In another example (Scheme 16), alcohol **75** was activated using triphosgene and triethylamine (or pyridine, or another organic base) and coupled to amine **33** [M. Tercel and W. A. Denny, *J. Chem. Soc. Perkin Trans. 1*, 1998, 509] to give carbamate **79** which was deprotected under acidic conditions to give **80**.

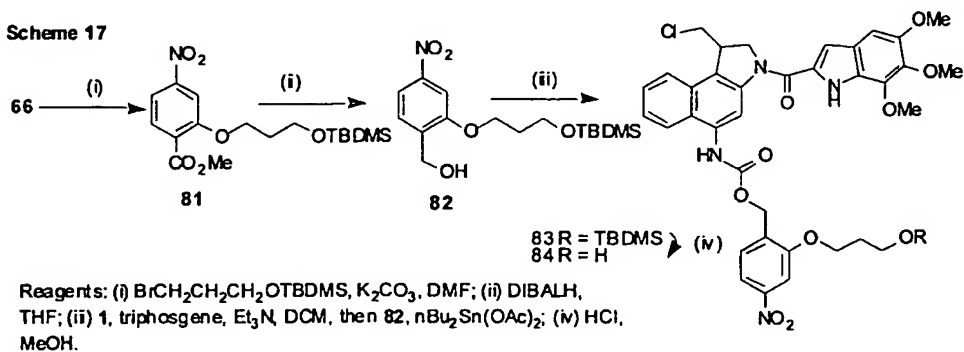
5

Scheme 16

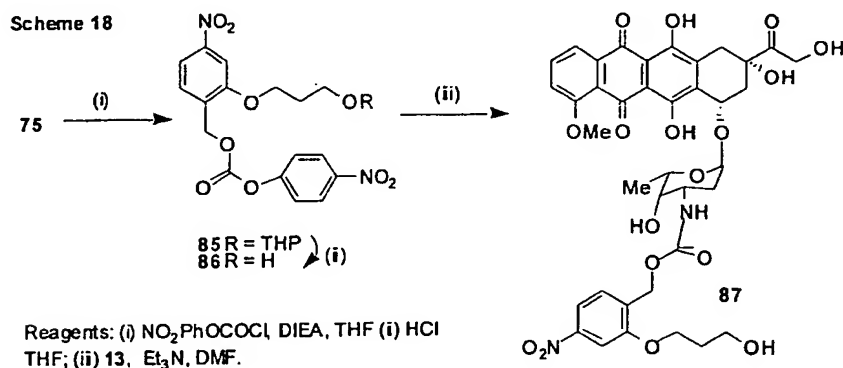


In another example (Scheme 17), alkylation of phenol **66** with 3-bromopropyl *tert*-butyl(dimethyl)silyl ether under basic conditions gave the ester **81** which was reduced to alcohol **82** using DIBALH in THF. Activation of amine **1** [G. J. Atwell, W. R. Wilson, W. A. Denny, *Bioorg. Med. Chem. Lett.*, 1997, 7, 1483] with triphosgene and triethylamine gave an intermediate isocyanate which was coupled with alcohol **82** using catalytic dibutyltin diacetate to give carbamate **83**. Deprotection of **83** under acidic conditions gave carbamate **84**.

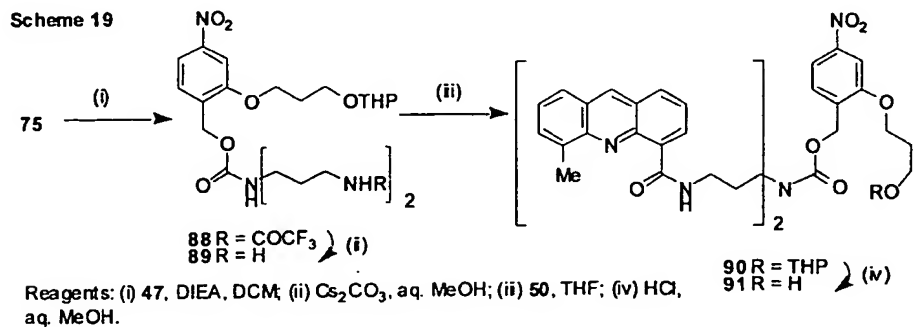
Scheme 17



In another example (Scheme 18), activation of alcohol **75** with 4-nitrophenylchloroformate gave the carbonate **37** which was deprotected under acidic conditions to give carbonate **86**. Reaction of **86** with doxorubicin (**13**) and triethylamine in DMF gave the doxorubicin carbamate **87**.

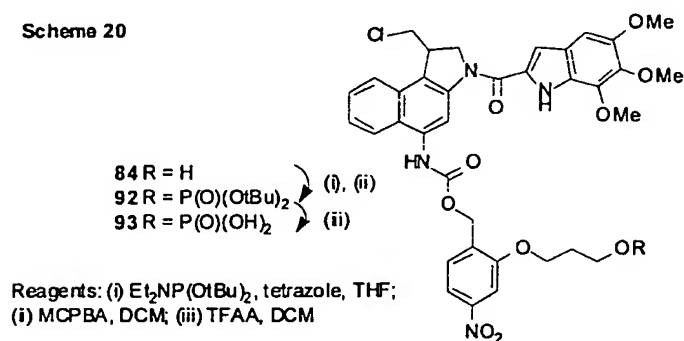


In another example (Scheme 19) alcohol **75** was activated with triphosgene and coupled to amine **47** to give trifluoroacetamide **88** which was deprotected under basic conditions to give bis-amine **89**. Coupling of the bis-amine **89** with the imidazolidine **50** [S. A. Gamage, J. A. Spicer, G. J. Atwell, G. J. Finlay, B. C. Baguley, W. A. Denny, *J. Med. Chem.*, 1999, 42, 2383-2393] gave the carbamate **90** which was deprotected under acidic conditions to give carbamate **91**.



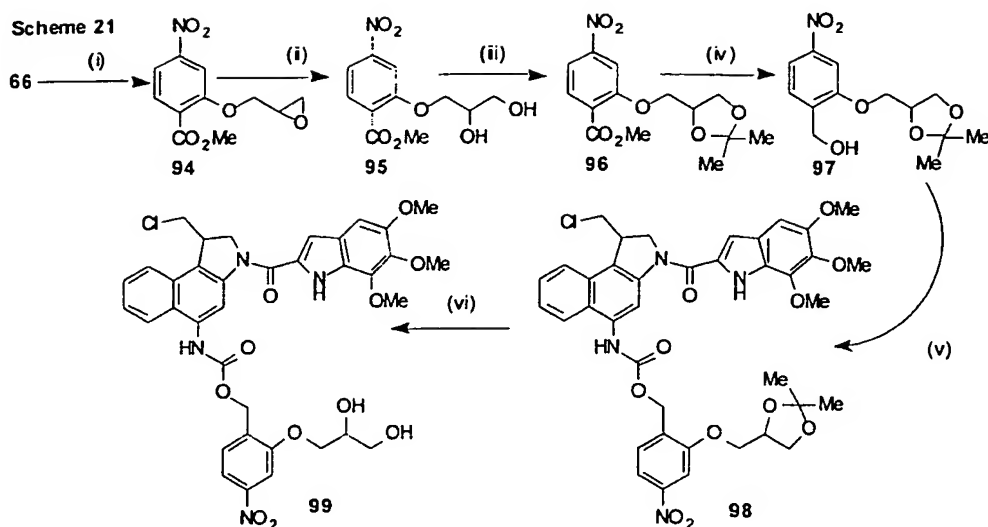
In another example (Scheme 20), the alcohol **84** was reacted with di-*tert*-butyl
 5 diethylphosphoramidite and tetrazole in THF and the intermediate oxidised with MCPBA to
 give ester **92**. Deprotection of **92** with trifluoroacetic acid (TFAA) gave the phosphate **93**.

Scheme 20



In another example (Scheme 21), phenol **66** was alkylated with epichlorohydrin
 10 under basic conditions to give epoxide **94**. Hydrolysis of **94** with perchloric acid gave diol **95**
 which was protected as the acetonide **96**. Reduction of **96** with DIBALH in THF gave the
 alcohol **97**. Activation of amine **1** [G. J. Atwell, W. R. Wilson, W. A. Denny, *Bioorg. Med.*
Chem. Lett., **1997**, 7, 1483] with triphosgene and triethylamine gave an intermediate
 isocyanate which was coupled with alcohol **97** using catalytic dibutyltin diacetate to give
 15 carbamate **98**. Deprotection of **98** under acidic conditions gave carbamate **99**.

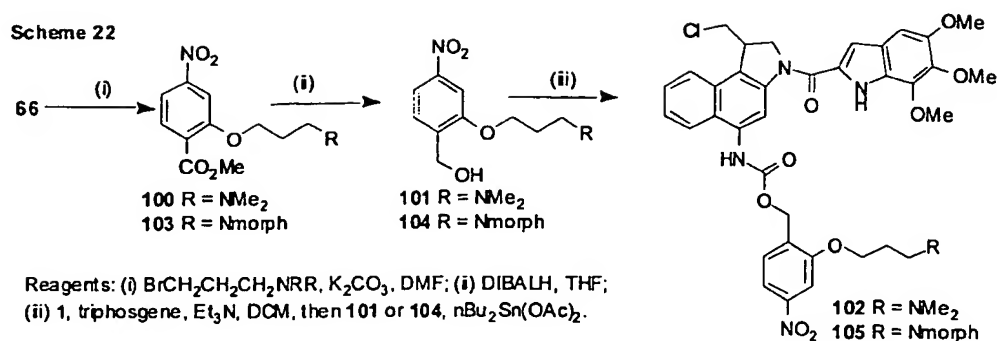
- 22 -



In another example (Scheme 22), phenol 66 was alkylated with *N*-(3-chloropropyl)-*N,N*-dimethylamine under basic conditions to give amine 100. Reduction of 100 with DIBALH in THF gave the alcohol 101. Activation of amine 1 [G. J. Atwell, W. R. Wilson, W. A. Denny, *Bioorg. Med. Chem. Lett.*, 1997, 7, 1483] with triphosgene and triethylamine gave an intermediate isocyanate which was coupled with alcohol 101 using catalytic dibutyltin diacetate to give carbamate 102.

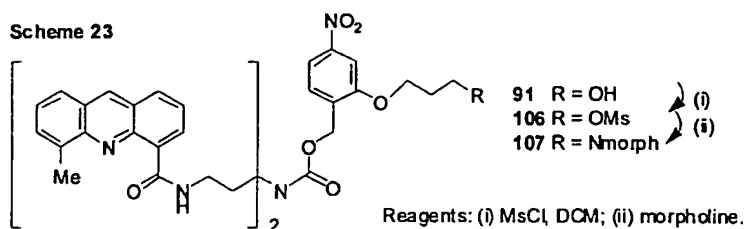
Similarly (Scheme 22), phenol 66 was alkylated with 4-(3-chloropropyl)morpholine under basic conditions to give amine 103. Reduction of 103 with DIBALH in THF gave the alcohol 104. Activation of amine 1 [G. J. Atwell, W. R. Wilson, W. A. Denny, *Bioorg. Med. Chem. Lett.*, 1997, 7, 1483] with triphosgene and triethylamine gave an intermediate isocyanate which was coupled with alcohol 104 using catalytic dibutyltin diacetate to give carbamate 105.

- 23 -



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In another example (Scheme 23), reaction of alcohol **91** with methanesulphonyl chloride gave the mesylate **106** which was reacted with morpholine to give carbamate **107**.



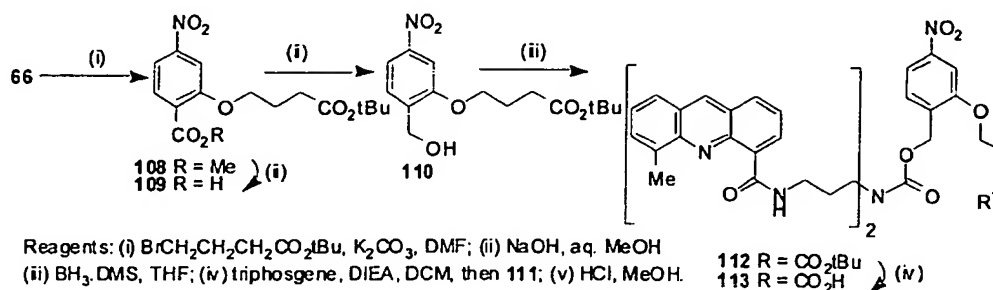
10

In another example (Scheme 24), phenol **66** was alkylated with *tert*-butyl 4-bromobutanoate under basic conditions to give ester **108**. Hydrolysis of **108** under basic conditions gave acid **109** which was reduced with borane.dimethylsulfide in THF to give alcohol **110**. Activation of alcohol **110** with triphosgene and diisopropylethylamine and subsequent coupling with *N,N*-bis[3-(5-methylacridine-4-carboxamido)propyl]amine (**111**) [S. A. Gamage, J. A. Spicer, G. J. Atwell, G. J. Finlay, B. C. Baguley, W. A. Denny, *J. Med.*

- 24 -

Chem., 1999, 42, 2383-2393] gave carbamate 112. Carbamate 112 was deprotected under acidic conditions to give acid 113.

Scheme 24

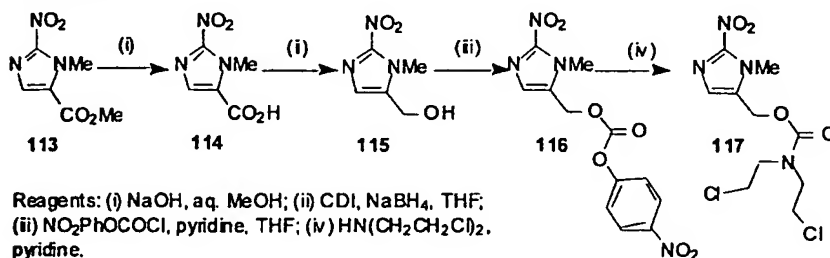


- 5 In a further aspect, the present invention relates to a method of preparing compounds of the general formula (II); examples of the methods are outlined in Schemes 25-.

Thus (Scheme 25), (2-nitro-1*H*-imidazol-5-yl)methanol (115) is obtained from the known ethyl 2-nitro-1*H*-imidazol-5-ylcarboxylate (113) [B. Cavalleri, R. Ballotta, G.C Lancini. *J. Heterocyclic Chem.* 1972, 9, 979.] by basic hydrolysis to the acid 114 and

- 10 reduction of an intermediate imidazolidine with sodium borohydride. This procedure is a major improvement upon the above published methods. Reaction of 115 with 4-nitrophenyl chloroformate gives the activated carbonate 116 which reacts with *N,N*-bis-(2-chloroethyl)amine to give carbamate 117.

Scheme 25

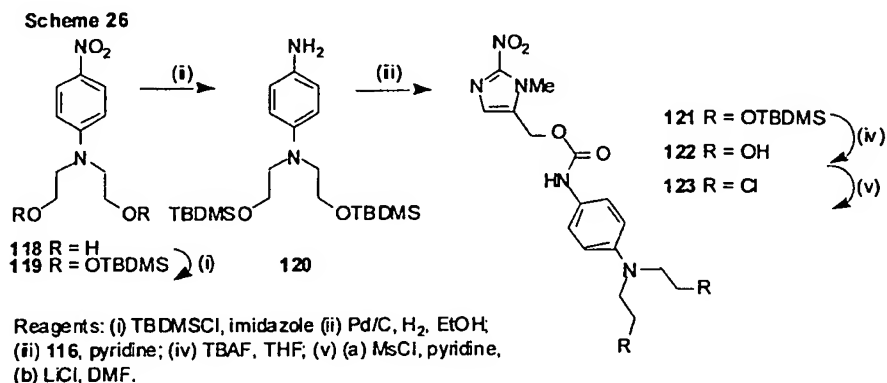


15

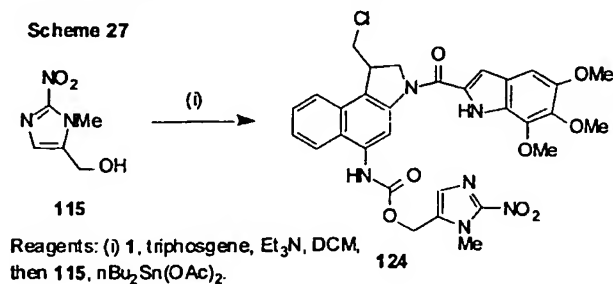
- 25 -

Similarly (Scheme 26), reaction of **116** with the protected phenyldiamine diol **120**, derived from the nitrophenylamino diol **118**, gives carbamate **121**. Deprotection of the bis-silyl alcohol **121** with TBAF gives the diol **122** which can be converted to the dichloride **123** under standard conditions.

5

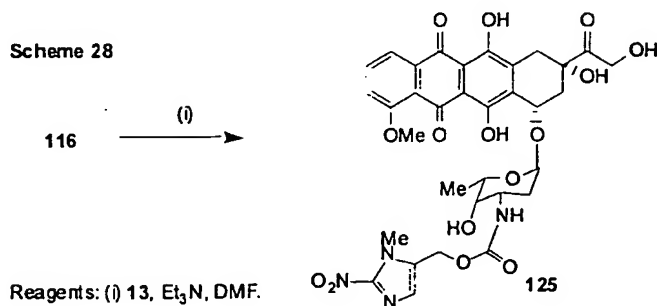


In another example (Scheme 27), activation of amine **1** [G. J. Atwell, W. R. Wilson,
 10 W. A. Denny, *Bioorg. Med. Chem. Lett.*, 1997, 7, 1483] with triphosgene and triethylamine (or
 pyridine, or another organic base) gave an intermediate isocyanate which was coupled with
 alcohol **115** using catalytic dibutyltin diacetate to give carbamate **124**.



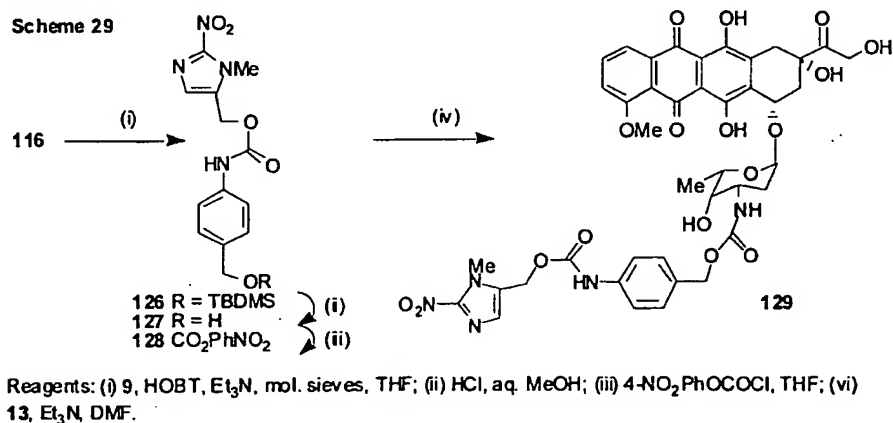
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In another example (Scheme 28), reaction of the carbonate **116** with doxorubicin (**13**) and triethylamine in DMF gave the carbamate **125**.



5

Similarly (Scheme 29), reaction of carbonate **116** with amine **9** using HOBT, molecular sieves, and triethylamine gave the silyl ether **126**. Deprotection of silyl ether **126** under acidic conditions gave alcohol **127** which was reacted with 4-nitrophenyl chloroformate to give carbonate **128**. Reaction of the carbonate **128** with doxorubicin (**13**) and triethylamine in DMF gave the carbamate **129**.

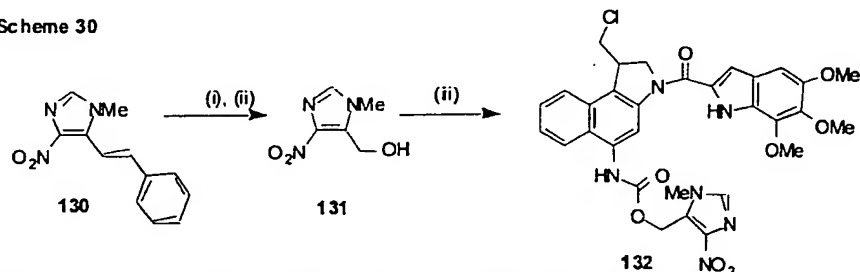


In another example (Scheme 30), ozonolysis of the styrene **130** [D. C. Baker, S. R. Putt, H. D. H. Showalter, *J. Heterocyclic Chem.*, **1983**, *30*, 629-634.] gave the alcohol **131**.

- 27 -

Activation of amine **1** [G. J. Atwell, W. R. Wilson, W. A. Denny, *Bioorg. Med. Chem. Lett.*, **1997**, *7*, 1483] with triphosgene and triethylamine gave an intermediate isocyanate which was coupled with alcohol **131** using catalytic dibutyltin diacetate to give carbamate **132**.

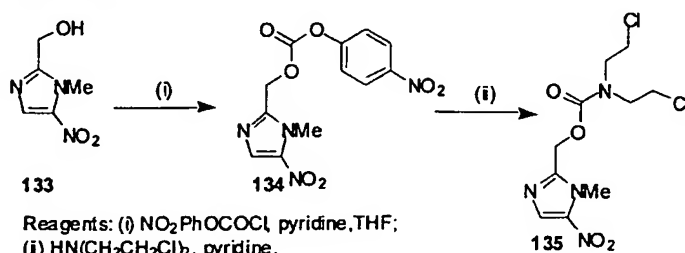
Scheme 30



Reagents: (i) O_3 , DCM, MeOH; (ii) $NaBH_4$, EtOH; (iii) **1**, triphosgene, Et_3N , DCM, then **131**, $nBu_2Sn(OAc)_2$.

In another example (Scheme 31), treatment of (N-methyl-5-nitro-1H-imidazol-2-yl)methanol (**133**) [C. Rufer, H. J. Kessler, E. Schroder, *J. Med. Chem.* 1971, *14*, 94.] with 4-nitrophenylchloroformate gave the carbonate **134**, which was displaced with N,N-bis(2-chloroethyl)amine to give the carbamate **135**.

Scheme 31

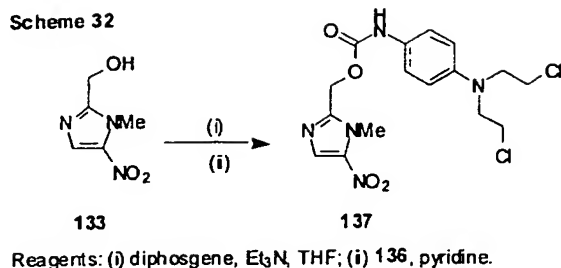


Reagents: (i) $NO_2PhOCOCl$, pyridine, THF; (ii) $HN(CH_2CH_2Cl)_2$, pyridine.

Similarly (Scheme 32), activation of **133** with diphosgene and subsequent reaction with N,N'-bis(2-chloroethyl)-1,4-benzenediamine hydrochloride (**136**) gave the carbamate **137**.

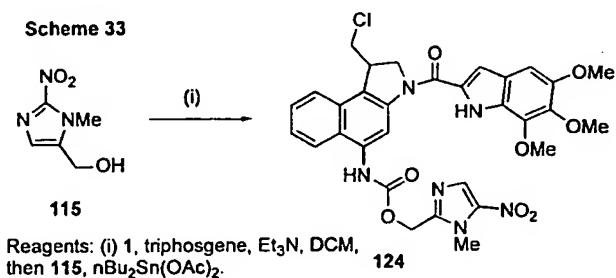
- 28 -

Scheme 32



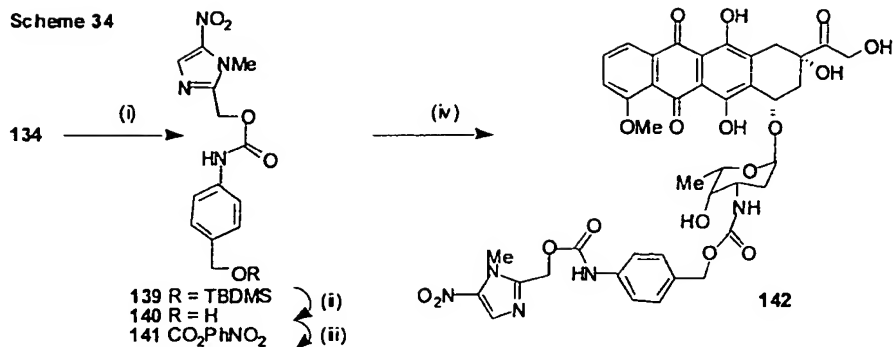
In another example (Scheme 33), activation of amine **1** [G. J. Atwell, W. R. Wilson, W. A. Denny, *Bioorg. Med. Chem. Lett.*, **1997**, *7*, 1483] with triphosgene and triethylamine gave an intermediate isocyanate which was coupled with alcohol **133** using catalytic dibutyltin diacetate to give carbamate **138**.

Scheme 33



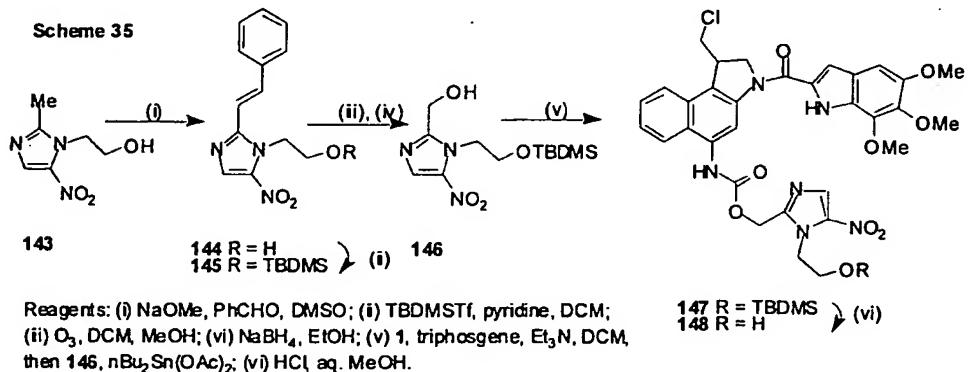
In another example (Scheme 34), reaction of carbonate **134** with amine **9** using HOBT, molecular sieves, and triethylamine gave the silyl ether **139**. Deprotection of silyl ether **139** under acidic conditions gave alcohol **140** which was reacted with 4-nitrophenyl chloroformate to carbonate **141**. Reaction of the carbonate **141** with doxorubicin (**13**) and triethylamine in DMF gave the carbamate **142**.

- 29 -

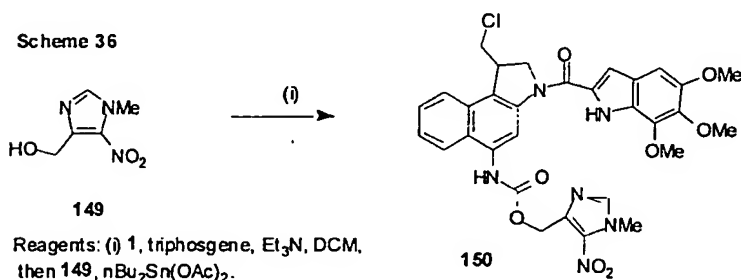


Reagents: (i) 9, HOBT, Et₃N, mol. sieves, THF; (ii) HCl, aq. MeOH; 4-NO₂PhOCOCN, THF; (vi) 13, Et₃N, DMF.

In another example (Scheme 35), condensation of metronidazole (143) and benzaldehyde gave the styrene 144 which was protected with TBDMS triflate to give 145. Ozonolysis of styrene 145 gave alcohol 146. Activation of amine 1 [G. J. Atwell, W. R. Wilson, W. A. Denny, *Bioorg. Med. Chem. Lett.*, 1997, 7, 1483] with triphosgene and triethylamine gave an intermediate isocyanate which was coupled with alcohol 146 using catalytic dibutyltin diacetate to give carbamate 147. Deprotection under acidic conditions gave the carbamate 148.



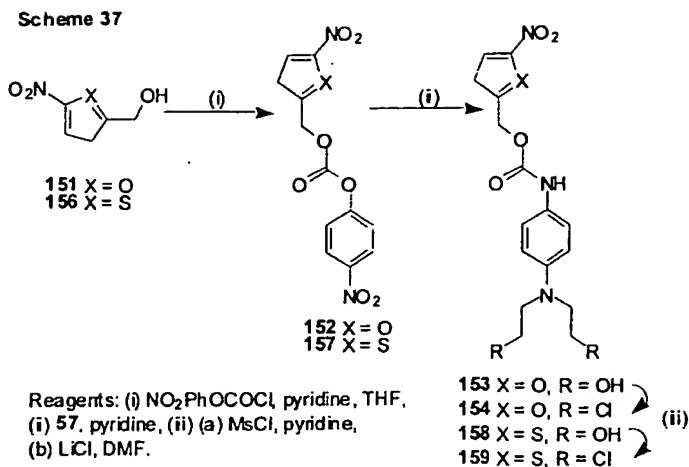
Similarly (Scheme 36), activation of amine **1** [G. J. Atwell, W. R. Wilson, W. A. Denny, *Bioorg. Med. Chem. Lett.*, **1997**, *7*, 1483] with triphosgene and triethylamine gave an intermediate isocyanate which was coupled with alcohol **149** [D. C. Baker, S.R. Putt, H. D. H. Showalter, *J. Heterocyclic Chem.*, **1983**, *20*, 629-634.] using catalytic dibutyltin diacetate to give carbamate **150**.



In another example (Scheme 37), reaction of the 5-nitrofuran-1-methanol (**151**) [J. M. Berry, C. Y. Watson, W. J. D. Wish, and M. D. Threadgill, *J. Chem. Soc. Perkin Trans. I*, **1997**, 1147.] with 4-nitrophenylchloroformate gave carbonate **152**, which was displaced with *N*¹,*N*¹-bis(2-hydroxyethyl)-1,4-benzenediamine (**57**) to give the carbamate diol **153**. The diol **153** was converted to the dichloride **154** using standard methods.

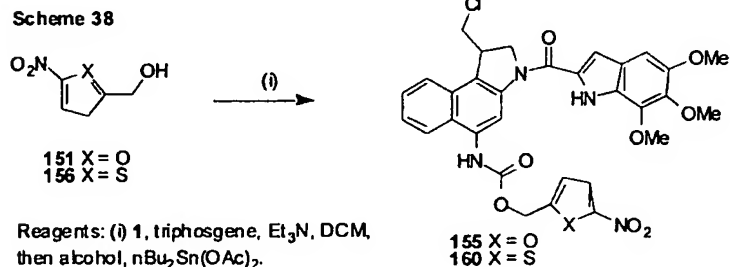
Similarly (Scheme 37), reaction of (5-nitrothien-2-yl)methanol (**156**) [P. J. Narcombe, R. K. Norris. *Aust. J. Chem.* **1979**, *32*, 2647] with 4-nitrophenylchloroformate gave carbonate **157**, which was displaced with *N*¹,*N*¹-bis(2-hydroxyethyl)-1,4-benzenediamine (**57**) to give the carbamate diol **158**. The diol **158** was converted to the dichloride **159** using standard methods. The same technique was used on 5-nitrofuran-1-methanol (**151**) [J.M. Berry, C.Y. Watson, W.J. Wish, and M.D. Threadgill, *J. Chem. Soc. Perkin Trans. I*, **1997**, 1147].

- 31 -



In another example (Scheme 38), activation of amine **1** [G. J. Atwell, W. R. Wilson, W. A. Denny, *Bioorg. Med. Chem. Lett.*, **1997**, *7*, 1483] with triphosgene and triethylamine gave an intermediate isocyanate which was coupled with alcohol **151** [J. M. Berry, C. Y. Watson, W. J. D. Whish, and M. D. Threadgill, *J. Chem. Soc. Perkin Trans. I*, **1997**, 1147.] using catalytic dibutyltin diacetate to give carbamate **155**.

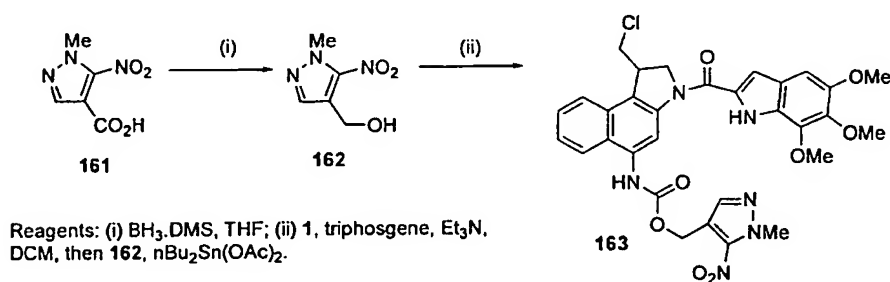
Similarly (Scheme 38), activation of amine **1** [G. J. Atwell, W. R. Wilson, W. A. Denny, *Bioorg. Med. Chem. Lett.*, **1997**, *7*, 1483] with triphosgene and triethylamine gave an intermediate isocyanate which was coupled with alcohol **156** [P. J. Narcombe, R. K. Norris, *Aust. J. Chem.*, **1979**, *32*, 2647] using catalytic dibutyltin diacetate to give carbamate **160**.



- 32 -

In another example (Scheme 39), 1-methyl-5-nitro-1*H*-pyrazole-4-carboxylic acid (161) [C. C. Cheng, *J. Heterocyclic Chem.* **1968**, *5*, 195-197] was reduced with borane.dimethyl sulfide to give alcohol 162. Activation of amine 1 [G. J. Atwell, W. R. Wilson, W. A. Denny, *Bioorg. Med. Chem. Lett.*, **1997**, *7*, 1483] with triphosgene and triethylamine gave an intermediate isocyanate which was coupled with alcohol 162 using catalytic dibutyltin diacetate to give carbamate 163.

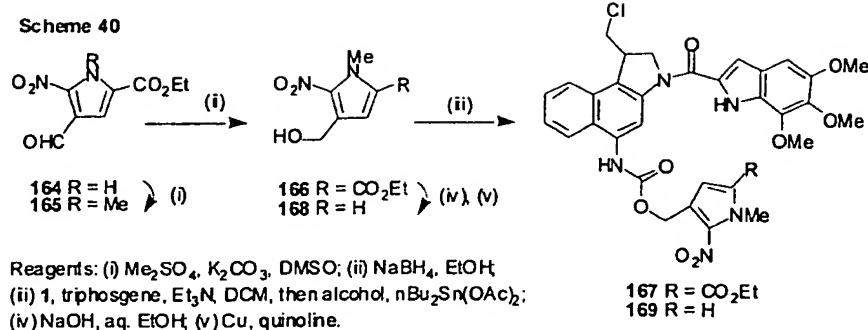
Scheme 39



In another example (Scheme 40), ethyl 4-formyl-5-nitro-1*H*-pyrrole-2-carboxylate (164) [P. Fornari, M. Farnier, C. Fournier, *Bull. Soc. Chim. Fr.* **1972**, 283-291] was alkylated with dimethyl sulfate to give pyrrole 165. Reduction of 165 with sodium borohydride gave the alcohol 166. Activation of amine 1 [G. J. Atwell, W. R. Wilson, W. A. Denny, *Bioorg. Med. Chem. Lett.*, **1997**, *7*, 1483] with triphosgene and triethylamine gave an intermediate isocyanate which was coupled with alcohol 166 using catalytic dibutyltin diacetate to give carbamate 167.

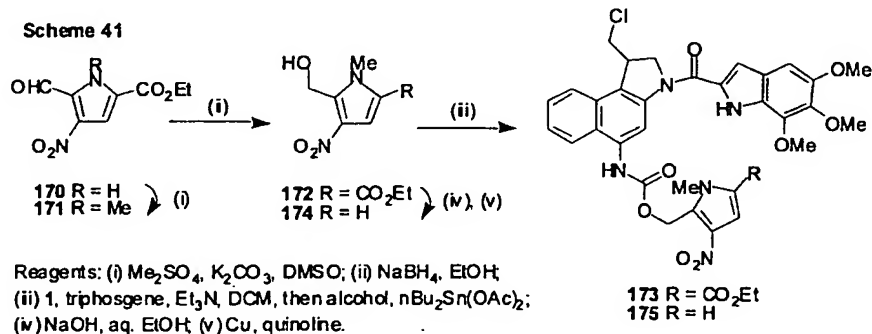
In another example (Scheme 40), hydrolysis of ester 166 followed by decarboxylation with copper in quinoline gave alcohol 168. Activation of amine 1 [G. J. Atwell, W. R. Wilson, W. A. Denny, *Bioorg. Med. Chem. Lett.*, **1997**, *7*, 1483] with triphosgene and triethylamine gave an intermediate isocyanate which was coupled with alcohol 168 using catalytic dibutyltin diacetate to give carbamate 169.

- 33 -

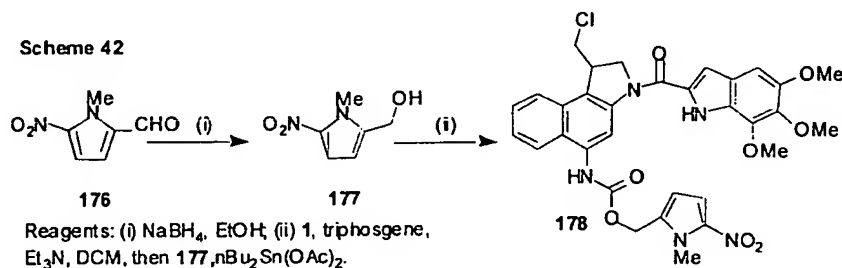


Similarly (Scheme 41), ethyl 5-formyl-4-nitro-1H-pyrrole-2-carboxylate (**170**) [P. Fornari, M. Farnier, C. Fournier, *Bull. Soc. Chim. Fr.* 1972, 283-291] was alkylated with dimethyl sulfate to give pyrrole **171**. Reduction of **171** with sodium borohydride gave the alcohol **172**. Activation of amine **1** [G. J. Atwell, W. R. Wilson, W. A. Denny, *Bioorg. Med. Chem. Lett.*, 1997, 7, 1483] with triphosgene and triethylamine gave an intermediate isocyanate which was coupled with alcohol **172** using catalytic dibutyltin diacetate to give carbamate **173**.

In another example (Scheme 41), hydrolysis of ester **172** followed by decarboxylation with copper in quinoline gave alcohol **174**. Activation of amine **1** [G. J. Atwell, W. R. Wilson, W. A. Denny, *Bioorg. Med. Chem. Lett.*, 1997, 7, 1483] with triphosgene and triethylamine gave an intermediate isocyanate which was coupled with alcohol **174** using catalytic dibutyltin diacetate to give carbamate **175**.



In another example (Scheme 42), 1-methyl-5-nitro-1*H*-pyrrole-2-carbaldehyde (**176**) [P. Fournari, *Bull. Soc. Chim. Fr.* **1963**, 488-491] was reduced with sodium borohydride to give alcohol **177**. Activation of amine **1** [G. J. Atwell, W. R. Wilson, W. A. Denny, *Bioorg. Med. Chem. Lett.*, **1997**, *7*, 1483] with triphosgene and triethylamine gave an intermediate isocyanate which was coupled with alcohol **177** using catalytic dibutyltin diacetate to give carbamate **178**.



In a further preferred aspect, the present invention relates to the use of a compound of formula (I) or (II) as defined in the second aspect of the invention, in conjunction with a nitroreductase enzyme (for example, isolated from *E. coli*) in a method of ADEPT or GDEPT therapy. A drug produced by the action of the nitroreductase enzyme on a compound of formula (I) or (II) may be used for the selective killing of oxic and hypoxic tumour cells in methods of treatment of cancers, for example leukemias and particularly solid cancers including breast, bowel and lung tumours, including small cell lung carcinoma.

The invention also provides a pharmaceutical composition comprising a compound of the formula (I) or (II) as defined in the second aspect of the invention together with a pharmaceutically acceptable carrier or diluent.

Detailed Description of the Invention

GDEPT

- Vector systems

In general, the vector for use in GDEPT therapies may be any suitable DNA or RNA vector.

Suitable viral vectors include those which are based upon a retrovirus. Such vectors are widely available in the art. Huber *et al.* (ibid) report the use of amphotropic retroviruses for the transformation of hepatoma, breast, colon or skin cells. Culver *et al.* (Science (1992)

256; 1550-1552) also describe the use of retroviral vectors in GDEPT. Such vectors or vectors derived from them may also be used. Other retroviruses may also be used to make vectors suitable for use in the present invention. Such retroviruses include rous sarcoma virus (RSV).

Englehardt *et al.* (Nature Genetics (1993) 4; 27-34) describe the use of adenovirus based vectors in the delivery of the cystic fibrosis transmembrane conductance product (CFTR) into cells, and such adenovirus based vectors may also be used. Vectors utilising adenovirus promoter and other control sequences may be of use in delivering a system according to the invention to cells in the lung, and hence useful in treating lung tumours.

Other vector systems including vectors based on the Molony murine leukaemia virus are known (Ram, Z *et al.* Cancer Research (1993) 53; 83-88; Dalton & Treisman, Cell (1992) 68; 597-612). These vectors contain the Murine Leukaemia virus (MLV) enhancer cloned upstream at a β -globin minimal promoter. The β -globin 5' untranslated region up to the initiation ATG is supplied to direct efficient translation of the enzyme.

Suitable promoters which may be used in vectors described above, include MLV, CMV, RSV and adenovirus promoters. Preferred adenovirus promoters are the adenovirus early gene promoters. Strong mammalian promoters may also be suitable. An example of such a promoter is the EF-1 α promoter which may be obtained by reference to Mizushima and Nagata ((1990), Nucl. Acids Res. 18; 5322). Variants of such promoters retaining substantially similar transcriptional activities may also be used.

20 - Nitroreductase

Compounds of the formula (I) or (II) can be activated by reduction of one (or more) of the available nitro groups by nitroreductase.

Preferably, the enzyme is a non-mammalian nitroreductase enzyme, such as a bacterial nitroreductase. An *E.coli* nitroreductase as disclosed in WO93/08288 is particularly preferred. The enzyme may be modified by standard recombinant DNA techniques, e.g. by cloning the enzyme, determining its gene sequence and altering the gene sequence by methods such as truncation, substitution, deletion or insertion of sequences for example by site-directed mutagenesis. Reference may be made to "Molecular Cloning" by Sambrook *et al.* (1989, Cold Spring Harbor) for discussion of standard recombinant DNA techniques. The modification made may be any which still leaves the enzyme with the ability to reduce the nitro group in formula I or II but alters other properties of the enzyme, for example its rate of reaction or selectivity.

In addition, small truncations in the N- and/or C-terminal sequence may occur as a result of the manipulations required to produce a vector in which a nucleic acid sequence encoding the enzyme is linked to the various other vector sequences.

ADEPT

- 5 For applications in ADEPT systems, an antibody directed against a tumour specific marker is linked to the nitroreductase enzyme, which may be modified as described above. The antibody may be monoclonal or polyclonal. For the purposes of the present invention, the term "antibody", unless specified to the contrary, includes fragments of whole antibodies which retain their binding activity for a tumour target antigen. Such fragments include Fv, 10 F(ab') and F(ab')₂ fragments, as well as single chain antibodies. Furthermore, the antibodies and fragments thereof may be humanised antibodies, e.g. as described in EP-A-239400.

- The antibodies may be produced by conventional hybridoma techniques or, in the case of modified antibodies or fragments, by recombinant DNA technology, eg by the expression in a suitable host vector of a DNA construct encoding the modified antibody or fragment 15 operably linked to a promoter. Suitable host cells include bacterial (eg. *E.coli*), yeast, insect and mammalian. When the antibody is produced by such recombinant techniques the enzyme may be produced by linking a nucleic acid sequence encoding the enzyme (optionally modified as described above) to the 3' or 5' end of the sequence of the construct encoding the antibody or fragment thereof.

20 Applications of the invention

- Compounds of the invention can be used *in vitro* or *in vivo* for a range of applications. For example, a number of vector systems for the expression of nitroreductase in a cell have been developed. The further development of such systems (e.g. the development of promoters suitable for specific cell types) requires suitable candidate prodrugs capable of killing cells 25 when activated by nitroreductase. Prodrug compounds of the present invention may be used in such model systems. The model systems may be *in vitro* model systems or xenograft model systems comprising for example human tumour cells implanted in nude mice.

- Compounds of the invention which are not activatable by an enzyme may be tested *in vitro* against panels of different tumour cells types to determine efficacy against such tumour 30 cells. The efficacy of compounds of the invention against a range of tumour cell types may be used as points of reference for the development of further antitumour compounds. Compounds of the present invention may also be tested in combination with additional anti-

cancer compounds to determine potential combination drug systems, for example combinations which are synergistic.

The compounds of the invention may also be used in a method of treatment of the human or animal body. Such treatment includes a method of treating the growth of neoplastic cells in a patient with neoplastic disease which comprises administering to a patient in need of treatment a compound of formula (I) or (II) of the invention as part of an ADEPT or GDEPT therapy system. Neoplastic diseases include leukaemia and solid tumours such as breast, bowel and lung tumours including small cell lung carcinoma.

It will be understood that where treatment of tumours is concerned, treatment includes any measure taken by the physician to alleviate the effect of the tumour on a patient. Thus, although complete remission of the tumour is a desirable goal, effective treatment will also include any measures capable of achieving partial remission of the tumour as well as a slowing down in the rate of growth of a tumour including metastases. Such measures can be effective in prolonging and/or enhancing the quality of life and relieving the symptoms of the disease.

15 ADEPT therapy

The antibody/enzyme conjugate for ADEPT can be administered simultaneously but it is often found preferable, in clinical practice, to administer the enzyme/agent conjugate before the prodrug, e.g. up to 72 hours or even 1 week before, in order to give the enzyme/agent conjugate an opportunity to localise in the region of the tumour target. By operating in this way, when the prodrug is administered, conversion of the prodrug to the cytotoxic agent tends to be confined to the regions where the enzyme/agent conjugate is localised, i.e. the region of the target tumour, and the premature release of the compound produced by the action of the nitroreductase on the compound of formula (I) or (II) is minimised.

In ADEPT the degree of localisation of the enzyme/agent conjugate (in terms of the ratio of localized to freely circulating active conjugate) can be further enhanced using the clearance and/or inactivation systems described in WO89/10140. This involves, usually following administration of the conjugate and before administration of the prodrug, the administration of a component (a "second component") which is able to bind to part of the conjugate so as to inactivate the enzyme and/or accelerate the clearance of the conjugate from the blood. Such a component may include an antibody to the enzyme component of the system which is capable of inactivating the enzyme.

The second component may be linked to a macromolecule such as dextran, a liposome,

albumin, macroglobulin or a blood group O erythrocyte so that the second component is restrained from leaving the vascular compartment. In addition or as an alternative, the second component may include a sufficient number of covalently bound galactose residues, or residues of other sugars such as lactose or mannose, so that it can bind the conjugate in plasma but be removed together with the conjugate from plasma by receptors for galactose or other sugars in the liver. The second component should be administered and designed for use such that it will not, to any appreciable extent, enter the extravascular space of the tumour where it could inactivate localised conjugate prior to and during administration of the prodrug.

In ADEPT systems, the dose of the prodrug and conjugate will ultimately be at the discretion of the physician, who will take into account such factors as the age, weight and condition of the patient. Suitable doses of prodrug and conjugate are given in Bagshawe *et al.* Antibody, Immunoconjugates, and Radiopharmaceuticals (1991), 4, 915-922. A suitable dose of conjugate may be from 500 to 200,000 enzyme units/m² (e.g. 20,000 enzyme units/m²) and a suitable dose of prodrug may be from about 0.1 to 200 mg/Kg, preferably about from 10 to 100 mg/Kg per patient per day.

In order to secure maximum concentration of the conjugate at the site of desired treatment, it is normally desirable to space apart administration of the two components by at least 4 hours. The exact regime will be influenced by various factors including the nature of the tumour to be targeted and the nature of the prodrug, but usually there will be an adequate concentration of the conjugate at the site of desired treatment within 48 hours.

The ADEPT system when used with nitroreductase also preferably comprises a suitable cofactor for the enzyme. Suitable cofactors include a riboside or ribotide of nicotinic acid or nicotinamide.

The antibody/enzyme conjugate may be administered by any suitable route usually used in ADEPT therapy. This includes parenteral administration of the antibody in a manner and in formulations similar to that described below.

GDEPT therapy

For use of the vectors in therapy, the vectors will usually be packaged into viral particles and the particles delivered to the site of the tumour, as described in for example Ram *et al.* (ibid). The viral particles may be modified to include an antibody, fragment thereof (including a single chain) or tumour-directed ligand to enhance targeting of the tumour. Alternatively the vectors may be packaged into liposomes. The liposomes may be targeted to

a particular tumour. This can be achieved by attaching a tumour-directed antibody to the liposome. Viral particles may also be incorporated into liposomes. The particles may be delivered to the tumour by any suitable means at the disposal of the physician. Preferably, the viral particles will be capable of selectively infecting the tumour cells. By "selectively
5 infecting" it is meant that the viral particles will primarily infect tumour cells and that the proportion of non-tumour cells infected is such that the damage to non-tumour cells by administration of a prodrug will be acceptably low, given the nature of the disease being treated. Ultimately, this will be determined by the physician.

One suitable route of administration is by injection of the particles in a sterile solution.
10 Viruses, for example isolated from packaging cell lines may also be administered by regional perfusion or direct intratumoral direction, or direct injection into a body cavity (intracavitary administration), for example by intra-peritoneum injection.

The exact dosage regime for GDEPT will, of course, need to be determined by individual clinicians for individual patients and this, in turn, will be controlled by the exact
15 nature of the prodrug and the cytotoxic agent to be released from the prodrug but some general guidance can be given. Chemotherapy of this type will normally involve parenteral administration of modified virus and administration by the intravenous route is frequently found to be the most practical.

In GDEPT systems the amount of virus or other vector delivered will be such as to
20 provide a similar cellular concentration of enzyme as in the ADEPT system mentioned above. Typically, the vector will be administered to the patient and then the uptake of the vector by transfected or infected (in the case of viral vectors) cells monitored, for example by recovery and analysis of a biopsy sample of targeted tissue. This may be determined by clinical trials which involve administering a range of trial doses to a patient and measuring the degree of
25 infection or transfection of a target cell or tumour. The amount of prodrug required will be similar to or greater than that for ADEPT systems.

In using a GDEPT system the prodrug will usually be administered following administration of the vector encoding an enzyme. Suitable doses of prodrug are from about 0.1 to 200 mg/Kg, preferably about from 10 to 100 mg/Kg per patient per day.

30 Administration of prodrug

While it is possible for a compound of formula (I) or (II) to be administered alone it is preferable to present it as a pharmaceutical formulation. Suitable formulations comprise the

compounds, together with one or more acceptable carriers thereof and optionally other therapeutic ingredients. The carrier or carriers must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not deleterious to the recipients thereof, for example, liposomes. Suitable liposomes include, for example, those comprising
5 the positively charged lipid (N[1-(2,3-dioleoyloxy)propyl]-N,N,N-triethylammonium (DOTMA), those comprising dioleoylphosphatidylethanolamine (DOPE), and those comprising 3β [N-(N',N'-dimethylaminoethane)-carbamoyl]cholesterol (DC-Chol).

Formulations suitable for parenteral or intramuscular administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers,
10 bacteriostats, bactericidal antibiotics and solutes which render the formulation isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents, and liposomes or other microparticulate systems which are designed to target the compound to blood components or one or more organs. The formulations may be presented in unit-dose or multi-dose containers,
15 for example sealed ampoules and vials, and may be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid carrier, for example water, for injections, immediately prior to use. Injection solutions and suspensions may be prepared extemporaneously from sterile powders, granules and tablets of the kind previously described.

It should be understood that in addition to the ingredients particularly mentioned above
20 the formulations may include other agents conventional in the art having regard to the type of formulation in question. Of the possible formulations, sterile pyrogen-free aqueous and non-aqueous solutions are preferred.

The doses may be administered sequentially, eg. at daily, weekly or monthly intervals, or in response to a specific need of a patient. Preferred routes of administration are oral
25 delivery and injection, typically parenteral or intramuscular injection or intratumoral injection.

The exact dosage regime will, of course, need to be determined by individual clinicians for individual patients and this, in turn, will be controlled by the exact nature of the compound of formula (I) or (II) but some general guidance can be given. Typical dosage ranges generally will be those described above which may be administered in single or
30 multiple doses. Other doses may be used according to the condition of the patient and other factors at the discretion of the physician.

The following Examples illustrate the invention.

General procedures

Analyses were carried out in the Microchemical Laboratory, University of Otago, Dunedin, NZ. Melting points were determined on an Electrothermal 2300 Melting Point Apparatus. IR spectra were recorded on a Midac FT-IR as KBr discs, unless otherwise stated. NMR spectra were obtained on a Bruker AM-400 spectrometer at 400 MHz for ^1H and 100 MHz for ^{13}C spectra. Spectra were obtained in CDCl_3 , unless otherwise specified, and are referenced to Me_4Si . Chemical shifts and coupling constants were recorded in units of ppm and Hz, respectively. Assignments were determined by APT, COSY, HSQC, and HMBC experiments. Mass spectra were determined on a VG-70SE mass spectrometer using an ionizing potential of 70 eV at a nominal resolution of 1000. High resolution spectra were obtained at nominal resolutions of 3000, 5000, or 10000 as appropriate. All spectra were obtained as electron impact (EI) using PFK as the reference unless otherwise stated. Solutions in organic solvents were dried with anhydrous Na_2SO_4 . Solvents were evaporated under reduced pressure on a Buchi rotary evaporator. Thin-layer chromatography was carried out on aluminium-backed silica gel plates (Merck 60 F_{254}) with visualisation of components by UV light (254 nm) or exposure to I_2 . Column chromatography was carried out on silica gel, (Merck 230-400 mesh). All compounds designated for biological testing were analyzed at >99% purity by reverse phase HPLC using a Philips PU4100 liquid chromatograph, a Phenomenex BondClone 10-C18 stainless steel column (300mm \times 3.9 mm i.d.) and a Philips PU4120 diode array detector. Chromatograms were run using various gradients of aqueous (1 M NaH_2PO_4 , 0.75 M heptanesulfonic acid, 0.5 M dibutylammonium phosphate, and MilliQ water in a 1:1:1:97 ratio) and organic (80% MeOH/MilliQ water) phases. DCM refers to dichloromethane; DIEA refers to diisopropylethylamine, DMF refers to dry dimethyl formamide; DMSO refers to dimethylsulphoxide; ether refers to diethyl ether; EtOAc refers to ethyl acetate; EtOH refers to ethanol; $i\text{Pr}_2\text{O}$ refers to diisopropyl ether; light petroleum refers to petroleum ether, boiling range 40-60 $^\circ\text{C}$; MeOH refers to methanol; THF refers to tetrahydrofuran dried over sodium benzophenone ketyl. All solvents were freshly distilled.

Example 1. Preparation of 4-nitrobenzyl 4-(1,3-benzothiazol-2-yl)phenylcarbamate (8). 4-Nitrophenyl chloroformate (0.15 g, 0.46 mmol) was added to a stirred solution of 2-

(4-aminophenyl)benzthiazole (7) [D-F. Shi, T.D. Bradshaw, S. Wrigley, C.J. McCall, P. Lelieveld, I. Fitchner, M.F.G. Stevens. *J. Med. Chem.*, 1996, 39, 3375] in pyridine (5 mL) and the solution stirred at 20 °C for 2 h. The solution was dilute with water (10 mL) and the mixture stirred for 40 min, filtered and the solid triturated with hot EtOH to give 8 (157 mg, 87%) as a pale green powder, mp 232-234 °C; ¹H NMR [(CD₃)₂SO]δ 10.31 (s, 1 H, OCONH), 8.29 (ddd, *J* = 8.7, 3.2, 2.2 Hz, 2 H, H 3", H 5"), 8.11 (d, *J* = 8.3 Hz, 1 H, H 4), 8.04 (br d, *J* = 8.7 Hz, 2 H, H 2", H 6"), 8.02 (d, *J* = 8.3 Hz, 1 H, H 7), 7.72 (br d, *J* = 8.7 Hz, 2 H, H 2', H 6'), 7.68 (br d, *J* = Hz, 2 H, H 3', H 5'), 7.51-7.55 (m, 1 H, H 5), 7.40-7.46 (m, 1 H, H 6), 5.32 (s, 2 H, CH₂O); Anal. (C₂₁H₁₅N₃O₄S) C, H, N.

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Example 2A. Preparation of 2-methoxy-4-nitrobenzyl 4-[bis(2-chloroethyl)amino]phenylcarbamate (32).

Methyl 2-methoxy-4-nitrobenzoate (28). An ethereal solution of diazomethane (CAUTION) was added to a solution of 4-nitrosalicylic acid (27) (1.0 g, 5.46 mmol) in ether (50 mL) until a yellow colour persisted and the solution stood at 20 °C for 4 h. The reaction was quenched with glacial acetic acid (2 mL), poured into sat. aq. NaHCO₃ solution and extracted with ether (2 × 50 mL). The combined organic fractions were dried and the solvent evaporated to give 28 (1.11 g, 96%) as white needles, mp (ether) 89-90 °C; IR N 1740, 1526, 1252, and 1086 cm⁻¹; ¹H NMR δ 7.89 (d, *J* = 8.3 Hz 1 H, H 5), 7.82-7.85 (m, 2 H, H 3, H 6), 4.01 (s, 3 H, OCH₃), and 3.94 (s, 3 H, OCH₃); ¹³C NMR 165.2 (CO₂), 159.2 (C 2), 150.7 (C 4), 132.0 (C 1), 126.0 (C 6), 115.0 (C 5), 106.9 (C 3), 56.6 (OCH₃), and 52.6 (OCH₃); Anal. (C₉H₉NO₅) C, H, N.

2-Methoxy-4-nitrobenzyl alcohol (29). A solution of 28 (0.9 g, 4.26 mmol) in THF (20 mL) was added dropwise to a stirred solution of DIBALH (1 M solution in toluene, 13.4 mL, 13.4 mmol) in THF (20 mL) at 2 °C and the solution stirred at 2 °C for 15 min. The solvent was evaporated and residue partitioned between EtOAc (100 mL) and water (100 mL). The aqueous fraction was extracted with EtOAc (2 × 50 mL) and the combined organic fraction dried and the solvent evaporated. The residue was purified by chromatography, eluting with 50% EtOAc/light petroleum, to give 29 (0.74 g, 93%) as cream needles, mp (EtOAc/light petroleum) 103-104 °C; IR n 3310, 1523, 1250, and 1036 cm⁻¹; ¹H NMR δ 7.86 (dd, *J* = 8.3, 2.1 Hz, 1 H, H 5), 7.71 (d, *J* = 2.1 Hz, 1 H, H 3), 7.52 (d,

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- 43 -

$J = 8.3$ Hz, 1 H, H 6), 4.76 (d, $J = 5.5$ Hz, 2 H, CH₂O), 3.96 (s, 3 H, OCH₃), and 2.27 (br s, 1 H, OH); ¹³C NMR δ 157.1, 148.3, 136.6, 127.9, 116.0, 105.0, 60.7, and 55.9; Anal. (C₈H₉NO₄) C, H, N.

- 5 **2-Methoxy-4-nitrobenzyl 4-[bis(2-hydroxyethyl)amino]phenylcarbamate (31).** Pyridine (91 μ L, 1.13 mmol) was added dropwise to a stirred solution of **29** (207 mg, 1.13 mmol) and triphosgene (117 mg, 0.40 mmol) in THF (10 mL) at 5 °C and the suspension stirred at 5 °C for 1 h. A solution of *N*¹,*N*¹-bis(2-hydroxyethyl)-1,4-benzenediamine (**57**) [prepared by catalytic hydrogenation of *N,N*-bis-(2-hydroxyethyl) 4-nitroaniline (**30**)] (244 mg, 1.24
- 10 mmol) with Pd/C under H₂ (60 psi) in EtOH) in THF (10 mL) and DMF (10 mL) was added and the mixture stirred at 20 °C for 16 h. The solvent was evaporated and the residue purified by chromatography, eluting with EtOAc to give **31** (251 mg, 55%) as orange prisms, mp (EtOAc) 153-154 °C; ¹H NMR δ 9.45 (br s, 1 H, OCONH), 7.90 (dd, $J = 8.3$, 2.0 Hz, 1 H, H 5'), 7.80 (d, $J = 2.0$ Hz, 1 H, H 3'), 7.60 (br d, $J = 8.3$ Hz, 1 H, H 6'), 7.21
- 15 (br d, $J = 9.0$ Hz, 2 H, H 2, H 6), 6.61 (d, $J = 9.0$ Hz, 2 H, H 3, H 5), 5.17 (s, 2 H, CH₂O), 4.71 (t, $J = 5.4$ Hz, 2 H, 2 \times OH), 3.97 (s, 3 H, OCH₃), 3.48-3.53 (m, 4 H, 2 \times CH₂O), 3.32-3.37 (m, 4 H, 2 \times CH₂N); ¹³C NMR δ 157.4, 153.7, 148.6, 144.6, 133.2, 129.0, 127.8, 120.7 (2), 116.0, 111.9 (2), 105.9, 60.6, 58.7 (2), 56.7, 53.9 (2); Anal. (C₁₉H₂₃N₃O₇) C, H, N.
- 20 **2-Methoxy-4-nitrobenzyl 4-[bis(2-chloroethyl)amino]phenylcarbamate (32).** Methanesulphonyl chloride (129 μ L, 1.67 mmol) was added dropwise to a stirred solution of **31** (226 mg, 0.55 mmol) in pyridine (10 mL) at 20 °C and the solution stirred for 1 h. The solvent was evaporated and the residue partitioned between DCM/water (100 mL). The aqueous fraction was extracted with DCM (2 \times 50 mL) and the combined organic fraction
- 25 washed with brine (50 mL), dried and the solvent evaporated. The residue was dissolved in DMF (10 mL), LiCl (0.15 g, 3.34 mmol) added, and the mixture stirred at 80 °C for 2 h. The solvent was evaporated and the residue partitioned between EtOAc/water (100 mL). The aqueous fraction was extracted with EtOAc (2 \times 25 mL). The combined organic fraction was washed with brine (30 mL), dried, and the solvent evaporated. The residue
- 30 was purified by chromatography, eluting with 25% EtOAc/light petroleum, to give **32** (230 mg, 93%) as pale green needles, mp (EtOAc/light petroleum) 129-130 °C; ¹H NMR δ 7.84 (dd, $J = 8.3$, 2.1 Hz, 1 H, H 5'), 7.72 (d, $J = 2.1$ Hz, 1 H, H 3'), 7.51 (d, $J = 8.3$ Hz, 1 H, H

- 44 -

6'), 7.27 (br d, $J = 9.0$ Hz, 2 H, H 2, H 6), 6.65 (ddd, $J = 9.0, 3.5, 2.1$ Hz, 2 H, H 3, H 5), 5.29, (s, 2 H, CH₂O), 3.96 (s, 3 H, OCH₃), 3.68-3.72 (m, 4 H, 2 × CH₂N), 3.58-3.63 (m, 4 H, 2 × CH₂Cl); ¹³C NMR δ 157.2, 153.4, 148.6, 142.9, 132.2, 128.5, 128.1, 121.4 (2), 115.7, 112.7 (2), 105.3, 61.3, 56.0, 53.7 (2), 40.5 (2); Anal. (C₁₉H₂₁Cl₂N₃O₅) C, H, N.

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Example 2B. Preparation of 2-methoxy-4-nitrobenzyl 3-(chloromethyl)-1-[(5,6,7-trimethoxy-1*H*-indol-2-yl)carbonyl]-2,3-dihydro-1*H*-indol-6-ylcarbamate (34).

Pyridine (20 μL, 0.25 mmol) was added dropwise to a stirred solution of 2-methoxy-4-nitrobenzyl alcohol (29) (45 mg, 0.25 mmol) and triphosgene (26 mg, 0.09 mmol) in THF (10 mL) at 5 °C and the suspension stirred at 5 °C for 1 h. A solution of 6-amino-3-(chloromethyl)-1-[(5,6,7-trimethoxyindol-2-yl)carbonyl]indoline (33) [M. Tercel and W. A. Denny. *J. Chem. Soc. Perkin Trans. 1*, 1998, 509] (102 mg, 0.25 mmol) in THF (10 mL) was added and the mixture stirred at 20 °C for 16 h. The suspension was filtered and the solvent evaporated. The residue was purified by chromatography, eluting with 40%EtOAc/DCM, to give 34 (102 mg, 65%) as a tan powder, mp (DCM/pet. ether) 144-150 °C; ¹H NMR δ 9.74 (s, 1 H, indole-NH), 8.26 (d, $J = 0.8$ Hz, 1 H, H 7), 7.62-7.68 (m, 3 H, H 5, H 3'', H 5''), 7.58 (br s, 1 H, OCONH), 7.35 (br d, $J = 8.1$ Hz, 1 H, H 6''), 7.20 (d, $J = 8.3$ Hz, 1 H, H 4), 6.91 (d, $J = 2.1$ Hz, 1 H, H 3'), 6.83 (s, 1 H, H 4'), 5.21 (s, 2 H, CH₂O), 4.58 (dd, $J = 10.6, 8.9$ Hz, 1 H, H 2), 4.41 (d, $J = 10.6, 4.3$ Hz, 1 H, H 2), 4.03, (s, 3 H, OCH₃), 3.92 (s, 3 H, OCH₃), 3.90 (s, 3 H, OCH₃), 3.89 (s, 3 H, OCH₃), 3.76-3.80 (m, 2 H, H 3, CH₂Cl), 3.51 (dd, $J = 11.7, 10.6$ Hz, 1 H, CH₂Cl); ¹³C NMR δ 160.5, 157.1, 153.1, 150.2, 148.4, 144.0, 140.5, 138.8, 138.7, 132.0, 129.5, 128.3, 126.0, 125.6, 124.5, 123.5, 115.6, 115.5, 108.8, 106.7, 105.5, 97.5, 61.4, 61.3, 61.1, 56.2, 55.9, 54.8, 46.9, 43.2; MS (FAB⁺) m/z 627 (MH⁺, 4%), 625 (MH⁺, 12), 234 (25), 149 (100); HRMS (FAB⁺) calc. for C₃₀H₃₀³⁵ClN₄O₁₀ (MH⁺) m/z 625.1701, found 625.1690; C₃₀H₃₀³⁷ClN₄O₁₀ (MH⁺) m/z 627.1672, found 627.1623; Anal. (C₃₀H₂₉ClN₄O₉) C, H, N.

Example 2C. Preparation of 2-methoxy-4-nitrobenzyl 1-(chloromethyl)-3-[(5,6,7-trimethoxy-1*H*-indol-2-yl)carbonyl]-2,3-dihydro-1*H*-benzo[*e*]indol-5-ylcarbamate (35). Phosgene (300 μL, 0.3 mmol, 1M in toluene) was added to a stirred solution of 2-methoxy-4-nitrobenzyl alcohol (29) (20 mg, 0.11 mmol) in THF (10 mL) and stirred at 20

- 45 -

°C for 16 h. The solvent was evaporated, the residue dissolved in THF (10 mL), a solution of amine 1 [G. J. Atwell, W. R. Wilson, W. A. Denny, *Bioorg. Med. Chem. Lett.*, **1997**, *7*, 1483] (50 mg, 0.11 mmol) in THF (10 mL) was added and the solution stirred at 20 °C for 4 days. The solvent was evaporated and the residue purified by chromatography, eluting with 50% EtOAc/light petroleum to give 35 (31 mg, 43%) as a tan solid, mp (EtOAc/light petroleum) 162-165 °C; ¹H NMR δ 9.52 (s, 1 H, indole-NH), 8.90 (s, 1 H, OCONH), 7.90 (d, *J* = 8.7 Hz, 1 H, H 6), 7.80 (d, *J* = 8.7 Hz, 1 H, H 5"), 7.77 (d, *J* = 8.4 Hz, 1 H, H 9), 7.70 (br s, 1 H, H 3"), 7.50-7.57 (m, 2 H, H 8, H 6"), 7.42-7.47 (m, 1 H, H 7), 7.25 (br s, 1 H, H 4), 6.99 (d, *J* = 2.2 Hz, 1 H, H 3'), 6.87 (s, 1 H, H 4'), 5.34 (d, *J* = 1.9 Hz, 2 H, CH₂O), 4.78 (dd, *J* = 10.7, 1.6 Hz, 1 H, H 2), 4.64 (dd, *J* = 10.7, 8.8 Hz, 1 H, H 2), 4.07-4.17 (m, 5 H, H 1, CH₂Cl, OCH₃), 3.95 (s, 3 H, OCH₃), 3.94 (s, 3H, OCH₃), 3.91 (s, 3 H, OCH₃), 3.45 (t, *J* = 10.9 Hz, 1 H, CH₂Cl); ¹³C NMR δ 160.3, 157.2, 154.0, 150.2, 148.6, 141.6, 140.6, 138.9, 133.9, 132.0, 129.7, 129.6, 128.8, 127.4, 127.2, 125.6, 125.4, 125.0, 123.6, 123.1, 123.0, 121.8, 122.4, 115.7, 106.5, 105.1, 97.6, 61.8, 61.5, 61.1, 56.2, 56.0, 54.9, 45.8, 43.4; MS (FAB⁺) *m/z* 675 (MH⁺, 10%), 677 (4), 659 (1), 639 (1), 517 (5), 234 (25); HRMS (FAB⁺) calc. for C₃₅H₃₂³⁵CIN₄O₉ (MH⁺) *m/z* 675.1858, found 674.1832; calc for C₃₅H₃₂³⁷CIN₄O₉ (MH⁺) *m/z* 677.1828, found 677.1834; Anal. (C₃₄H₃₁CIN₄O₉·H₂O) C, H, N.

Example 2D. Preparation of 2-methoxy-4-nitrobenzyl 4-(1,3-benzothiazol-2-yl)phenylcarbamate (36). Pyridine (36 mL, 0.44 mmol) was added dropwise to a stirred solution of alcohol of 2-methoxy-4-nitrobenzyl alcohol (29) (81 mg, 0.44 mmol) and triphosgene (66 mg, 0.22 mg) in DCM (10 mL) and the mixture was stirred at 20 °C for 20 min. A solution of 2-(4-aminophenyl)benzthiazole (7) [D-F. Shi, T. D. Bradshaw, S. Wrigley, C. J. McCall, P. Lelieveld, I. Fitchner, M. F. G. Stevens. *J. Med. Chem.*, **1996**, *39*, 3375] (100 mg, 0.44 mmol) in DCM (5 mL) and the mixture stirred at 20 °C for 4 h. The mixture was partitioned between EtOAc (100 mL) and sat. aq. KHCO₃ solution (50 mL), the organic fraction dried and the solvent evaporated. The residue was slurried in warm EtOAc/MeOH (1:1, 20 mL), filtered and the solvent evaporated to give 36 (123 mg, 64%) as a pale green powder mp (EtOH) 213-214 °C; ¹H NMR [(CD₃)₂SO] δ 10.31 (s, 1 H, OCONH), 8.11 (d, *J* = 7.8 Hz, 1 H, H 4), 8.01-8.06 (m, 3 H, H 7, H 2', H 6'), 7.92 (dd, *J* = 8.3, 2.2 Hz, 1 H, H 5"), 7.81 (d, *J* = 2.2 Hz, 1 H, H 3"), 7.65-7.69 (m, 3 H, H 3', H 5' H 6"),

- 46 -

7.51-7.55 (m, 1 H, H 5), 7.40-7.46 (m, 1 H, H 6), 5.27 (s, 2 H, CH₂O) 3.98 (s, 3 H, OCH₃); ¹³C NMR [(CD₃)₂SO] δ 166.9, 157.1, 153.6, 152.9, 148.3, 141.8, 134.2, 132.0, 129.0 (2), 128.1, 127.0, 126.5, 125.1, 122.4, 122.1, 118.3 (2), 115.5, 105.5, 60.7, 56.2; Anal. (C₂₂H₁₇N₃O₅S) C, H, N.

5

Example 2E. Preparation of 2-methoxy-4-nitrobenzyl doxorubicin carbamate (38).

2-Methoxy-4-nitrobenzyl 4-nitrophenyl carbonate (37). A solution of 4-nitrophenyl chloroformate (1.00 g, 4.97 mmol) in pyridine (4 mL) was added dropwise to a stirred solution of 2-methoxy-4-nitrobenzyl alcohol (29) (617 mg, 3.31 mmol) in pyridine (15 mL) at 20 °C and the solution stirred for 16 h. The solvent was evaporated and the residue purified by chromatography, eluting with a gradient (20-50%) EtOAc/light petroleum, to give 37 (928 mg, 80%) as pale yellow solid, mp (EtOAc/light petroleum) 105-106 °C; ¹H NMR δ 8.28 (ddd, *J* = 9.2, 3.1, 2.1 Hz, 2 H, H 3'), 7.89 (dd, *J* = 8.3, 2.1 Hz, 1 H, H 5), 7.77 (d, *J* = 2.1 Hz, 1 H, H 3), 7.58 (d, *J* = 8.3 Hz, 1 H, H 6), 7.40 (ddd, *J* = 8.3, 3.1, 2.1 Hz, 2 H, H 2'), 5.41 (s, 2 H, CH₂O), 4.00 (s, 3 H, OCH₃); ¹³C NMR δ 157.6 (C 2), 155.4 (OCO₂), 152.3 (C 1), 149.2 (C 4), 145.5 (C 4'), 129.8 (C 1), 129.3 (C 6), 125.3 (C 2'), 121.7 (C 3'), 115.8 (C 5), 105.5 (C 3), 65.3 (CH₂O), 56.2 (OCH₃); Anal. (C₁₅H₁₂N₂O₈) C, H, N.

2-Methoxy-4-nitrobenzyl doxorubicin carbamate (38). A solution of carbonate 37 (23 mg, 66 μmol) in DMF (2 mL) was added to a solution of doxorubicin (13) (30 mg, 55 μmol) and Et₃N (9 mL 66 μmol) in DMF (5 mL) at 20 °C and the solution stirred for 16 h. The solvent was evaporated and the residue purified by chromatography, eluting with a gradient (0-5%) of MeOH/DCM, to give 38 (37 mg, 88%) as a red solid, mp (DCM) 159-161 °C; ¹H NMR δ 13.97 (s, 1 H, 6-OH), 13.22 (s, 1 H, 11-OH), 8.02 (dd, *J* = 8.0, 1.0 Hz, 1 H, H 1), 7.77-7.81 (m, 2 H, H 2, H 5'), 7.66 (br s, 1 H, H 3'), 7.41 (d, *J* = 8.0 Hz, 1 H, H 6'), 7.39 (dd, *J* = 8.0, 1.0 Hz, 1 H, H 3), 5.52 (br d, *J* = 3.3 Hz, 1 H, H 1'), 5.29 (br s, 1 H, H 7), 5.25 (d, *J* = 8.7 Hz, 1 H, OCONH), 5.13 (2 d, *J* = 14.0 Hz, 2 H, CH₂O), 4.75 (s, 2 H, H 14), 4.51 (s, 1 H, 9-OH), 4.13-4.17 (m, 1 H, H 5'), 4.08 (s, 3 H, 4-OCH₃), 3.90 (s, 3 H, 2'-OCH₃), 3.84-3.88 (m, 1 H, H 3'), 3.69 (s, 1 H, H 4'), 3.24 (dd, *J* = 18.9, 1.3 Hz, 1 H, H 10), 3.03 (s, 1 H, 14-OH), 3.01 (d, *J* = 18.9 Hz, 1 H, H 10), 2.34 (br d, *J* = 14.7 Hz, 1 H, H 8), 2.18 (dd, *J* = 14.7, 4.0 Hz, 1 H, H 8), 2.02 (br s, 1 H, 4'-OH), 1.90 (dd, *J* = 13.2, 4.7 Hz, 1 H, H 2'), 1.79 (dd, *J* = 13.2, 3.3 Hz, 1 H, H 2'), 1.30 (d, *J* = 6.5 Hz, 3 H, H 6'); ¹³C NMR

- 47 -

δ 213.7 (C 13), 187.1 (C 5), 186.7 (C 12), 161.0 (C 4), 157.0 (C 2'), 156.1 (C 6), 155.6 (C 11), 155.2 (OCONH), 148.5 (C 4''), 135.8 (C 2), 135.5 (C12a), 133.5 (C 6a), 133.4 (C 10a), 132.5 (C 1'), 128.4 (C 6''), 120.8 (C 4a), 119.9 (C 1), 118.5 (C 3), 115.7 (C 5'), 111.6 (C 5a), 111.4 (C 11a), 105.1 (C 3''), 100.7 (C 1'), 76.6 (C 9), 69.8 (C 7), 69.6 (C 4'), 67.2 (C 5'), 65.5 (C 14), 61.1 (CH₂O), 56.7 (4-OCH₃), 56.0 (2'-OCH₃), 47.1 (C 3'), 35.6 (C 8), 34.0 (C 10), 30.2 (C 2), 16.8 (C 6'); MS (FAB⁺) *m/z* 753 (MH⁺, 0.3%); HRMS (FAB⁺) calc. for C₃₆H₃₇N₂O₁₆ (MH⁺) *m/z* 753.2143, found 753.2100; Anal (C₃₆H₃₆N₂O₁₆) C, H, N.

Example 2F. Preparation of 4-(((2-methoxy-4-

10 nitrobenzyl)oxy]carbonyl}amino)benzyl doxorubicin carbamate (42).

2-Methoxy-4-nitrobenzyl 4-(((*tert*-butyl(dimethyl)silyl)oxy}methyl)phenylcarbamate (39). Et₃N (0.40 mL, 2.84 mmol) was added to a stirred suspension of carbonate 37 (0.90 g, 2.58 mmol), 4-(((*tert*-butyl(dimethyl)silyl)oxy}methyl)aniline (9) (0.64 g, 2.71 mmol), HOBT (0.35 g, 2.58 mmol), and 4 Å molecular sieves (900 mg) in THF (80 mL) and the mixture stirred at 20 °C for 16 h. The solvent was evaporated and the residue partitioned between EtOAc (100 mL) and water (100 mL). The organic fraction was washed with 1 M HCl (2 × 40 mL), water (100 mL), brine (50 mL), dried, and the solvent evaporated. The residue was purified by chromatography, eluting with 20% EtOAc/light petroleum, to give 39 (0.89 g, 77%) as a white solid, mp (EtOAc/light petroleum) 120-122 °C; ¹H NMR δ 7.84 (dd, *J* = 8.3, 2.1 Hz, 1 H, H 5'), 7.72 (d, *J* = 2.1 Hz, 1 H, H 3'), 7.51 (d, *J* = 8.3 Hz, 1 H, H 6'), 7.35 (d, *J* = 8.3 Hz, 2 H, H 2, H 6), 7.26 (d, *J* = 8.3 Hz, 2 H, H 3, H 5), 6.76 (br s, 1 H, OCONH), 5.30 (s, 2 H, CH₂O), 4.69 (s, 2 H, CH₂OSi), 3.93 (s, 3 H, OCH₃), 0.92 (s, 9 H, SiC(CH₃)₃), 0.09 (s, 6 H, Si(CH₃)₂); ¹³C NMR δ 157.3 (C 2'), 153.0 (OCONH), 148.7 (C 4'), 137.0 (C 4), 136.4 (C 1), 132.1 (C 1'), 128.7 (C 6'), 126.9 (C 3, C 5), 118.6 (C 2, C 6), 115.7 (C 5'), 105.2 (C 3'), 64.6 (CH₂O), 61.4 (CH₂O), 56.0 (OCH₃), 26.9 (SiC(CH₃)₃), 18.4 (Si(CH₃)₂), -5.2 (Si(CH₃)₂); Anal. (C₂₂H₃₀N₂O₆Si) C, H, N.

2-Methoxy-4-nitrobenzyl 4-(hydroxymethyl)phenylcarbamate (40). 1 M HCl (4 mL, 4 mmol) was added to a stirred solution of silyl ether 39 (0.89 g, 0.2 mmol) in MeOH (10 mL) and stirred at 20 °C for 1 h. The solution was poured into brine (50 mL) and extracted with EtOAc (3 × 50 mL). The combined organic fraction was washed with water (50 mL), dried, and the solvent evaporated. The residue was purified by chromatography, eluting

- 48 -

with a gradient (20-50%) EtOAc/light petroleum, to give **40** (628 mg, 95%) as a white solid, mp (EtOAc/light petroleum) 164-165 °C; ¹H NMR [(CD₃)₂SO] δ 9.83 (br s, 1 H, OCONH), 7.90 (dd, *J* = 8.3, 2.1 Hz, 1 H, H 5'), 7.80 (d, *J* = 2.1 Hz, 1 H, H 3'), 7.63 (d, *J* = 8.3 Hz, 1 H, H 6'), 7.41 (d, *J* = 8.4 Hz, 2 H, H 2, H 6), 7.22 (d, *J* = 8.4 Hz, 2 H, H 3, H 5), 5.21 (s, 2 H, CH₂O), 5.07 (t, *J* = 5.6 Hz, 1 H, OH), 4.41 (t, *J* = 5.6 Hz, 2 H, CH₂O), 3.97 (s, 3 H, OCH₃); ¹³C NMR [(CD₃)₂SO] δ 157.0 (C 2'), 153.0 (OCONH), 148.2 (C 4'), 137.4 (C 4), 136.7 (C 1), 132.3 (C 1'), 128.8 (C 6'), 127.0 (C 3, C 5), 117.9 (C 2, C 6), 115.5 (C 5'), 105.4 (C 3'), 62.5 (CH₂O), 60.4 (CH₂O), 56.0 (OCH₃); Anal. (C₁₆H₁₆N₂O₆) C, H, N.

- 10 **4-(((2-Methoxy-4-nitrobenzyl)oxy)carbonyl)amino)benzyl 4-nitrophenyl carbonate (41)**. A solution of 4-nitrophenylchloroformate (205 mg, 1.02 mmol) in THF (5 mL) was added dropwise to a stirred solution of alcohol **40** (282 mg, 0.85 mmol) and Et₃N (142 μL, 1.02 mmol) in THF/DMF (1:1, 30 mL) the solution stirred at 20 °C for 16 h. The solvent was evaporated and the residue was purified by chromatography, eluting with 10% EtOAc/DCM, to give **41** (238 mg, 56 %) as a white powder mp (EtOAc/DCM) 144-146 °C; ¹H NMR [(CD₃)₂SO] δ 10.01 (s, 1 H, OCONH), 8.31 (ddd, *J* = 9.1, 3.4, 2.2 Hz, 2 H, H 3, H 5), 7.91 (dd, *J* = 8.3, 2.2 Hz, 1 H, H 5''), 7.81 (d, *J* = 2.2 Hz, 1 H, H 3''), 7.64 (d, *J* = 8.3 Hz, 1 H, H 6''), 7.56 (ddd, *J* = 9.1, 3.4, 2.2 Hz, 2 H, H 2, H 6), 7.53 (br d, *J* = 8.6 Hz, 2 H, H 3', H 5'), 7.41 (br d, *J* = 8.6 Hz, 2 H, H 2', H 6'), 5.24 (s, 4 H, 2 × CH₂O), 3.98 (s, 3 H, OCH₃); ¹³C NMR [(CD₃)₂SO] δ 157.0 (C 2''), 155.2 (OCO₂), 153.0 (OCONH), 151.9 (C 1), 148.2 (C 4''), 145.1 (C 4), 139.4 (C 1), 132.2 (C 1'), 129.6 (C 2', C 6'), 128.9 (C 6''), 128.5 (C 4'), 125.3 (C 2, C 6), 122.6 (C 3, C 5), 118.0 (C 3', C 5'), 115.5 (C 5''), 105.5 (C 3''), 70.2 (CH₂O), 60.5 (CH₂O), 56.2 (OCH₃); MS (FAB⁺) *m/z* 498 (MH⁺, 0.5%); HRMS (FAB⁺) calc. for C₂₃H₂₀N₃O₁₀ (MH⁺) *m/z* 498.1149, found 498.1151. Anal. (C₂₃H₁₉N₃O₁₀) C, H, N.

- 4-(((2-Methoxy-4-nitrobenzyl)oxy)carbonyl)amino)benzyl doxorubicin carbamate (42)**. A solution of carbonate **41** (52 mg, 103 μmol) in DMF (2 mL) was added dropwise to a stirred solution of doxorubicin (**13**) (45 mg, 86 μmol) and Et₃N (15 μL, 103 μmol) in DMF (5 mL) at 20 °C and the solution stirred at 20 °C for 24 h. The solvent was evaporated and the residue purified by chromatography, eluting with a gradient (0-5%) of MeOH/DCM, to give **42** (61 mg, 80%) as a red solid, mp (DCM) 128-131 °C; ¹H NMR

- 49 -

$[(\text{CD}_3)_2\text{SO}]$ δ 14.01 (s, 1 H, 6-OH), 13.25 (s, 1 H, 11-OH), 9.88 (s, 1 H, OCONH), 7.87-7.90 (m, 3 H, H 1, H 2, H 5'''), 7.79 (d, $J = 2.2$ Hz, 1 H, H 3'''), 7.59-7.63 (m, 2 H, H 3, H 6'''), 7.41 (d, $J = 8.3$ Hz, 2 H, H 3'', H 5''), 7.22 (d, $J = 8.3$ Hz, 2 H, H 2'', H 6''), 6.81 (d, $J = 8.0$ Hz, 1 H, OCONH), 5.44 (s, 1 H, H 7), 5.21 (d, $J = 3.0$ Hz, 1 H, H 1'), 5.19 (s, 2 H, CH₂O), 4.91-4.94 (m, 1 H, 9-OH), 4.87 (s, 2 H, CH₂O), 4.83 (dd, $J = 6.3, 5.9$ Hz, 1 H, 14-OH), 4.69 (d, $J = 5.7$ Hz, 1 H, 4-OH), 4.58 (d, $J = 6.0$ Hz, 2 H, H 14), 4.12-4.18 (m, 1 H, H 5'), 3.97 (s, 3 H, OCH₃), 3.95 (s, 3 H, OCH₃), 3.68-3.75 (m, 1 H, H 3'), 3.43-3.47 (m, 1 H, H 4'), 2.99 (d, $J = 18.4$ Hz, 1 H, H 10), 2.92 (d, $J = 18.4$ Hz, 1 H, H 10), 2.20 (br d, $J = 14.1$ Hz, 1 H, H 8), 2.12 (dd, $J = 14.1$ Hz, 1 H, H 8), 1.85 (dt, $J = 12.8, 3.7$ Hz, 1 H, H 2'), 1.47 (dd, $J = 12.8, 4.1$ Hz, 1 H, H 2'), 1.13 (d, $J = 6.5$ Hz, 3 H, H 6'); ¹³C NMR $[(\text{CD}_3)_2\text{SO}]$ δ 213.7 (C 13), 186.4 (C 5), 186.3 (C 12), 160.7 (C 4), 157.0 (C 2'''), 156.0 (C 6), 155.2 (C 11), 154.4 (OCONH), 152.9 (OCONH), 148.2 (C 4'''), 138.4 (C 4''), 136.1 (C 2), 135.4 (C 12a), 134.6 (C 6a), 134.0 (C 10a), 132.2 (C 1''), 131.0 (C 1'''), 128.9 (C 2'''), 128.6 (C 2'', C 6''), 119.9 (C 4a), 119.6 (C 1), 118.9 (C 3), 117.9 (C 3'', C 5''), 115.4 (C 5'''), 110.7 (C 5a), 110.6 (C 11a), 105.4 (C 3'''), 100.2 (C 1'), 74.9 (C 9), 69.8 (C 7), 67.9 (C 4'), 66.6 (C 5'), 64.8 (C 14), 63.6 (CH₂O), 60.4 (CH₂O), 56.5 (OCH₃), 56.2 (OCH₃), 47.0 (C 3'), 36.5 (C 8), 32.0 (C 10), 29.7 (C 2'), 16.9 (C 6'); MS (FAB⁺) m/z 902 (MH⁺, 0.2%); Anal. (C₄₄H₄₃N₃O₁₈·H₂O) C, H, N, calc 4.6, found 5.6%.

Example 2G. Preparation of 2-methoxy-4-nitrobenzyl 2-[(9-amino-5-methyl-4-acridinyl)carbonyl]amino)ethyl(methyl)carbamate (46).

2-Methoxy-4-nitrobenzyl methyl{2-[(trifluoroacetyl)amino]ethyl}carbamate (43). A solution of 2-methoxy-4-nitrobenzyl alcohol (29) (183 mg, 1.0 mmol) and DIEA (0.19 mL, 1.2 mmol) in DCM (2 mL) was added dropwise to a solution of triphosgene (104 mg, 0.35 mmol) in DCM (1.5 mL) over 30 min at °C. The reaction was stirred at 5 °C for 1 h, then a solution of 2,2,2-trifluoro-*N*-[2-(methylamino)ethyl]acetamide trifluoroacetate (282 mg, 1.0 mmol) and DIEA (0.38 mL, 2.4 mmol) in DCM (2 mL) was added and the solution stirred at 20 °C for 16 h. The solvent was evaporated and the residue purified by chromatography, eluting with 40% EtOAc/petroleum ether, to give 43 (371 mg, 94%) as a white solid, mp 107-109 °C; ¹H NMR δ 7.86 (dd, $J = 8.4, 2.2$ Hz, 1 H, H 5), 7.72 (d, $J = 2.2$ Hz, 1 H, H 3), 7.56 (br s, 1 H, CONH), 7.44 (d, $J = 8.4$ Hz, 1 H, H 6), 5.23 (s, 2 H, CH₂O), 3.95 (s, 3 H, OCH₃), 3.58 (br s, 4 H, 2 CH₂N), 3.03 (s, 3 H, NCH₃); ¹³C NMR δ 157.7 (C 2), 157.4 (q, J

- 50 -

= 37 Hz, $\underline{\text{COCF}_3}$), 157.1 (OCON), 148.6 (C 4), 132.2 (C 1), 128.1 (C 6), 115.7 (C 5), 115.7 (q, $J = 288$ Hz, CF_3), 105.2 (C 3), 62.3 (CH_2O), 56.0 (OCH_3), 47.9 (CH_2N), 39.5 (CH_2N), 35.0 (NCH_3); Anal. ($\text{C}_{14}\text{H}_{16}\text{F}_3\text{N}_3\text{O}_6$) C, H, N.

5 **2-Methoxy-4-nitrobenzyl 2-aminoethyl(methyl)carbamate (44).** A solution of carbamate 43 (948 mg, 2.5 mmol), Cs_2CO_3 (4.0 g, 12.0 mmol) and water (5 mL) in methanol (20 mL) was stirred at 20 °C for 8 h. The pH was adjusted to 10 with 1 M HCl, water (50 mL) was added, and the solution was extracted with DCM (3 50 mL). The combined organic phase was dried and the solvent was evaporated to give 44 (578 mg, 83%) as a colorless oil which
10 was used directly, ^1H NMR δ 7.85 (dd, $J = 2.0, 8.2$ Hz, 1 H, H 5), 7.72 (d, $J = 2.0$ Hz, 1 H, H 3), 7.47 (d, $J = 8.2$ Hz, 1 H, H 6), 5.23 (s, 2 H, CH_2O), 3.95 (s, 3 H, OCH_3), 3.37-3.40 (m, 2 H, CH_2), 2.99-3.02 (m, 3 H, CH_3N), 2.87-2.90 (m, 2 H, CH_2), NH_2 not observed; ^{13}C NMR δ 153.0, 148.5, 132.9, 128.3, 127.8, 115.7, 105.1, 61.7, 56.0, 52.2, 40.1, 35.2.

15 **2-Methoxy-4-nitrobenzyl 2-[(9-chloro-5-methyl-4-acridinyl)carbonyl]aminoethyl(methyl)carbamate (45).** A stirred suspension of 5-methyl-9-oxo-9,10-dihydro-4-acridinecarboxylic acid (16) (507 mg, 2.0 mmol) in SOCl_2 (30 mL) containing DMF (2 drops) was heated gently under reflux until homogeneous and then for a further 45 min. The solution was evaporated below 40 °C, and the residue
20 azeotroped with benzene. The residue was dissolved in DCM (30 mL), cooled to 5 °C, DIEA (1 mL, 6 mmol) and 44 added, and the solution stirred at 20 °C for 30 min. The solvent was evaporated and the residue purified by chromatography, eluting with 75% EtOAc/light petroleum, to give 45 (255 mg, 50 %) as a yellow solid, mp (EtOAc/light petroleum) 60-65°C; ^1H NMR [$(\text{CD}_3)_2\text{SO}$] δ 11.92-11.96 (m, 1 H, NH), 8.91-9.00 (m, 1 H),
25 8.53-8.61 (m, 1 H), 8.26-8.29 (m, 1 H), 7.66-7.75 (m, 2 H), 7.51-7.60 (m, 2 H), 7.17-7.30 (m, 2 H), 5.15 (s, 2 H, CH_2O), 3.89 (s, 3 H, OCH_3), 3.72-3.75 (m, 2 H, CH_2), 3.62-3.65 (m, 2 H, CH_2), 3.09 (s, 3 H, CH_3N), 2.75 (s, 3 H, CH_3); ^{13}C NMR [$(\text{CD}_3)_2\text{SO}$] δ 165.9, 156.3, 146.6, 145.0, 143.3, 135.9, 135.6, 132.4, 131.6, 128.8, 128.7, 128.4, 128.2, 127.7, 127.5, 126.5, 124.2, 123.8, 123.0, 115.5, 104.3, 61.5, 55.8, 49.0, 38.0, 35.2, 18.9.

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2-Methoxy-4-nitrobenzyl 2-[(9-amino-5-methyl-4-acridinyl)carbonyl]aminoethyl(methyl)carbamate (46). A solution of chloride 45 (100

mg, 0.17 mmol) in dry phenol (1.2 g, 13 mmol) was heated at 50 °C. Dry ammonia was bubbled through the solution while the temperature was raised from 50 to 120°C. Addition of ammonia was continued for 15 min, then the mixture was cooled and diluted with excess 40% aqueous NaOH. Prolonged cooling gave a solid that was purified by chromatography, 5 eluting with 20% MeOH/DCM, to give **46** (80 mg, 92 %) as a yellow solid, mp (MeOH/DCM) 245-248 °C; ¹H NMR [(CD₃)₂SO] δ 12.72-12.74 (m, 1 H, NH), 8.55-8.66 (m, 2 H), 8.28-8.24 (m, 1 H), 8.10-8.14 (m, 2 H), 7.66-7.09 (m, 4 H), 5.00 (s, 2 H, CH₂O), 3.73 (s, 3 H, OCH₃), 3.65 (m, 4 H, 2 CH₂), 2.95 (s, 3 H, CH₃N), 2.51 (s, 3 H, CH₃); ¹³C NMR [(CD₃)₂SO] δ 165.9, 155.7, 155.2, 152.2, 147.1, 146.2, 145.4, 134.3, 134.0, 132.2, 10 130.9, 127.2, 126.8, 126.6, 121.7, 121.1, 120.4, 114.7, 113.0, 111.7, 104.4, 60.8, 55.7, 48.4, 36.7, 34.3, 18.5. Anal. (C₂₇H₁₇N₅O₆) C, H, N.

Example 2H. Preparation of 2-methoxy-4-nitrobenzyl bis(3-[(5-methyl-4-acridinyl)carbonyl]amino)propyl)carbamate (51).

15 **2,2,2-Trifluoro-N-[3-({3-[(trifluoroacetyl)amino]propyl}amino)propyl]acetamide trifluoroacetate (47).** Water (1.2 mL, 70 mmol) was added to a stirred solution of *N*-(3-aminopropyl)-1,3-propanediamine (4.0 g, 30.5 mmol) and ethyl trifluoroacetate (15.0 g, 105 mmol) in MeCN (60 mL) and the solution heated at reflux for 3 h. The solution was cooled, the solvent evaporated, and the residue was triturated with DCM (100 mL). The 20 suspension was filtered to give **47** (1.20 g, 90%) as white solid, mp (DCM) 175-178 °C; ¹H NMR [(CD₃)₂SO] δ 9.55 (s, 2 H, NH₂⁺), 8.45 (br s, 2 H, 2 CONH), 3.24-3.28 (m, 4 H, 2 CH₂N), 2.90-2.94 (m, 4 H, 2 CH₂N), 1.76-1.84 (m, 4 H, 2 CH₂); Anal. (C₁₂H₁₆F₉N₃O₄) C, H, N.

25 **2-Methoxy-4-nitrobenzyl 3-[(trifluoroacetyl)amino]propyl(6,6,6-trifluoro-5-oxohexyl)carbamate (48).** A solution of 2-methoxy-4-nitrobenzyl alcohol (**29**) (183 mg, 1.0 mmol) and DIEA (0.19 mL, 1.2 mmol) in DCM (2 mL) was added dropwise to a solution of triphosgene (104 mg, 0.35 mmol) in DCM (1.5 mL) over 30 minutes at 5 °C and the solution stirred for 1 h. The solution was added dropwise to a suspension of 30 bistrifluoroacetamide **47** (437 mg, 1.0 mmol) and DIEA (0.38 mL, 2.4 mmol) in DCM (2 mL) and the solution stirred at 20 °C for 16 h. The solvent was evaporated and the residue purified by chromatography, eluting with 40% EtOAc/light petroleum, to give **48** (373 mg,

- 52 -

70%) as a white solid, mp (EtOAc/light petroleum) 133-135°C; ¹H NMR [(CD₃)₂SO] δ 9.41 (s, 2 H, 2 CONH), 7.85 (dd, *J* = 8.0, 2.0 Hz, 1 H, H 5), 7.78 (d, *J* = 2.0 Hz, 1 H, H 3), 7.50 (d, *J* = 8.0 Hz, 1 H, H 6), 5.12 (s, 2 H, CH₂O), 3.95 (s, 3 H, OCH₃), 3.15-3.35 (m, 8 H, 4 CH₂N), 1.74 (m, 4 H, 2 CH₂). Anal. (C₁₉H₂₂F₆N₄O₇) C, H, N.

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2-Methoxy-4-nitrobenzyl bis(3-[(5-methyl-4-acridinyl)carbonyl]amino)propyl)carbamate (51). A solution of carbamate **48** (107 mg, 0.2 mmol), Cs₂CO₃ (1.0 g, 3.0 mmol) and water (1 mL) in methanol (4 mL) was stirred at 20 °C for 8 hrs. The pH was adjusted to 10 with 1M HCl, water (50 mL) added, and the solution was extracted with DCM (3 × 50 mL). The combined organic fraction was dried, the solvent was evaporated to give crude 2-methoxy-4-nitrobenzyl bis(3-aminopropyl)carbamate **49**. 4-(1*H*-Imidazol-1-ylcarbonyl)-5-methylacridine (**50**) [S. A. Gamage, J. A. Spicer, G. J. Atwell, G. J. Finlay, B. C. Baguley, W. A. Denny, *J. Med. Chem.*, **1999**, *42*, 2383-2393] (104 mg, 0.36 mmol) was added to a solution of the crude carbamate **49** in THF (10 mL) at 5 °C and the reaction mixture was stirred at 20 °C for 8 hrs. The solvent was evaporated, and the residue was purified by chromatography on alumina-90, eluting with 2%MeOH/45%EtOAc/DCM, to give **51** (85 mg, 64%) as a yellow solid, mp (EtOAc/DCM) 88-90 °C; ¹H NMR δ 11.87 (s, 1 H, NH), 11.81 (s, 1 H, NH), 8.90 (s, 2 H), 8.70 (s, 1 H), 8.67 (s, 1 H), 8.03 (m, 2 H), 7.78 (m, 2 H), 7.58 (m, 4 H), 7.40 (m, 2 H), 7.28 (d, *J* = 2.0 Hz, 1 H, H 3'''), 7.05 (d, *J* = 8.4 Hz, 1 H, H 6'''), 6.97 (dd, *J* = 8.4, 2.0 Hz, 1 H, H 5'''), 5.01 (s, 2 H, CH₂O), 3.71 (m, 7 H), 3.58 (m, 4 H), 2.81 (s, 3 H, CH₃), 2.70 (s, 3 H, CH₃), 2.11 (m, 4 H); ¹³C NMR δ 166.1, 156.3, 155.6, 147.8, 146.9 (2), 146.7, 145.2, 145.1, 137.8, 135.7 (2), 135.3, 135.1 (2), 132.3 (2), 132.2 (2), 130.9, 128.2, 127.9, 126.9 (2), 126.5, 126.3, 126.2, 126.1 (2), 125.7 (2), 125.3 (2), 115.1, 104.2, 61.6, 55.6, 45.8, 45.1, 37.7, 37.1, 29.2, 28.5, 18.9, 18.7; Anal. (C₄₅H₄₂N₆O₇·½H₂O) C, H, N.

Example 2I. Preparation of 2-methoxy-4-nitrobenzyl 2-[(5,8-dihydroxy-4-[(2-methyl[(2-methoxy-4-nitrobenzyl)oxy]carbonyl]amino)ethyl]amino}-9,10-dioxo-9,10-dihydro-1-anthracenyl)amino]ethyl(methyl)carbamate (53). A solution of 1,4-difluoro-5,8-dihydroxyanthracene-9,10-dione (**22**) (1.0 g, 3.6 mmol) and 2-methoxy-4-nitrobenzyl 2-aminoethyl(methyl)carbamate (**44**) (0.8 g, 2.7 mmol) in pyridine (20 mL) was stirred at 20 °C for 48 h. The solvent was evaporated and residue was purified by

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column chromatography, eluting with a gradient (50-85%) of EtOAc/DCM, to give:

(i) starting material (22) (0.15 g, 15%) and:

(ii) 2-methoxy-4-nitrobenzyl 2-[(4-fluoro-5,8-dihydroxy-9,10-dioxo-9,10-dihydro-1-anthracenyl)amino]ethyl(methyl)carbamate (52) (0.54 g, 37%) as a purple solid mp

5 (EtOAc/DCM) 122-125 °C; ¹H NMR δ 13.05-13.77 (m, 2 H, 2 × OH), 9.84-10.07 (br d, 1 H, NH), 6.99-7.83 (m, 7 H), 5.27 (s, 2 H, CH₂O), 3.95 (s, 3 H, OCH₃), 3.63 (m, 4 H), 3.09 (s, 3 H, NCH₃); HRMS (FAB⁺) calc. for C₂₆H₂₂FN₃O₉ (M⁺) *m/z* 539.1340, found 539.1331; and:

10 (iii) 53 (120 mg, 5%) as a blue solid, ¹H NMR δ 13.15-13.36 (m, 2 H, 2 × OH), 10.29-10.42 (m, 2 H, 2 × NH), 6.97-7.82 (m, 10 H), 5.26 (s, 2 H, CH₂O), 5.12 (s, 2 H, CH₂O), 3.95 (s, 3 H, OCH₃), 2.78 (s, 3 H, OCH₃), 3.50 (br, 8 H), 3.05 (s, 6 H, 2 × NCH₃); HRMS (FAB⁺) calc. for C₃₈H₃₈N₆O₁₄ (M⁺) *m/z* 802.2446, found 802.2446.

Example 2J. Preparation of 2-methoxy-4-nitrobenzyl 2-[[5,8-dihydroxy-4-((2-[(2-hydroxyethyl)amino]ethyl)amino)-9,10-dioxo-9,10-dihydro-1-anthracenyl]amino]ethyl(methyl)carbamate (55).

A solution of fluoride 52 (0.54 g, 1.0 mmol) and 2-(2-aminoethylamino)ethanol (2.0 g, 19 mmol) in pyridine (20 mL) was stirred at 20 °C for 54 h. The solvent was evaporated and residue was purified by column chromatography, eluting with a gradient (50-85%) of

20 EtOAc/light petroleum followed by (2-10%) MeOH/EtOAc, to give:

(i) 2-methoxy-4-nitrobenzyl 2-[[8,11-dihydroxy-4-(2-hydroxyethyl)-7,12-dioxo-1,2,3,4,7,12-hexahydronaphtho[2,3-*f*]quinoxalin-6-yl]amino]ethyl(methyl)carbamate (54) (0.1 g, 16%) as a blue solid; mp (DCM/light petroleum) 221-224 °C; ¹H NMR δ 13.43-

14.25 (m, 2 H, 2 × OH), 10.89-11.29 (m, 2 H, 2 × NH), 6.96-7.77 (m, 6 H), 6.20 (s, 1 H), 4.80 (s, 2 H, CH₂O), 3.94 (s, 3 H, OCH₃), 3.65 (m, 12 H), 2.97 (s, 3 H, NCH₃); and:

25 (ii) 55 (0.45 g, 72%) as a blue oil, ¹H NMR δ 13.26-13.51 (m, 2 H, 2 × OH), 10.44-10.50 (m, 2 H, 2 × NH), 7.02-7.88 (m, 7 H), 5.28 (s, 2 H, CH₂O), 3.96 (s, 3 H, OCH₃), 3.39-3.65 (m, 12 H), 3.07 (s, 3 H, NCH₃), NH, OH not observed; HRMS (FAB⁺) calc. for C₃₀H₃₄N₅O₁₀ (MH⁺) *m/z* 624.2306, found 624.2297..

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Example 3A. Preparation of 1-(4-nitrophenyl)ethyl 4-[bis(2-chloroethyl)amino]phenylcarbamate (59).

- 54 -

1-(4-Nitrophenyl)ethyl 4-[bis(2-hydroxyethyl)amino]phenylcarbamate (**58**). Pyridine (320 mL, 3.95 mmol) was added dropwise to a stirred solution of 1-(4-nitrophenyl)ethanol **56** (660 mg, 3.95 mmol) and triphosgene (410 mg, 1.38 mmol) in THF (50 mL) at 5 °C and the suspension stirred at 5 °C for 1 h. A solution of *N*¹,*N*¹-bis(2-hydroxyethyl)-1,4-benzenediamine (**57**) (prepared by catalytic hydrogenation of *N,N*-bis-(2-hydroxyethyl)-4-nitroaniline (**30**) (0.85 g, 4.34 mmol) with Pd/C under H₂ (60 psi) in EtOH) in THF (10 mL) and DMF (20 mL) was added and the mixture stirred at 20 °C for 16 h. The solvent was evaporated and the residue purified by chromatography, eluting with EtOAc to give **58** (1.01 g, 66 %) as a brown oil, ¹H NMR δ 8.19 (d, *J* = 8.8 Hz, 2 H, H 3', H 5'), 7.52 (br d, *J* = 8.8 Hz, 2 H, H 2', H 6'), 7.16 (d, *J* = 9.0 Hz, 2 H, H 2, H 6), 6.69 (br s, 1 H, OCONH), 6.62 (d, *J* = 9.0 Hz, 2 H, H 3, H 5), 5.90 (q, *J* = 6.6 Hz, 1 H, CHO), 3.76-3.80 (m, 4 H, 2 × CH₂O), 3.55 (br s, 2 H, 2 × OH), 3.48-3.51 (m, 4 H, 2 × CH₂N), 1.58 (d, *J* = 6.6 Hz, 3 H, CH₃); ¹³C NMR δ 153.1, 149.4, 147.4, 144.9, 127.3, 126.6 (2), 123.8 (2), 121.4 (2), 113.3 (2), 71.9, 60.6 (2), 55.3 (2), 22.5; MS (FAB⁺) *m/z* 390 (MH⁺, 25%); HRMS (FAB⁺) calc. for C₁₉H₂₄N₃O₆ (MH⁺) *m/z* 390.1665, found 390.1656.

1-(4-nitrophenyl)ethyl 4-[bis(2-chloroethyl)amino]phenylcarbamate (**59**). Methanesulphonyl chloride (600 µL, 7.7 mmol) was added dropwise to a stirred solution of **58** (1.0 g, 2.57 mmol) in pyridine (20 mL) at 20 °C and the solution stirred for 1 h. The solvent was evaporated and the residue partitioned between DCM/water (100 mL). The aqueous fraction was extracted with DCM (2 × 50 mL) and the combined organic fraction washed with brine (50 mL), dried and the solvent evaporated. The residue was dissolved in DMF (15 mL), LiCl (0.65 g, 15.4 mmol) added, and the mixture stirred at 80 °C for 3 h. The solvent was evaporated and the residue partitioned between EtOAc/water (200 mL). The aqueous fraction was extracted with EtOAc (2 × 50 mL). The combined organic fraction was washed with brine (50 mL), dried, and the solvent evaporated. The residue was purified by chromatography, eluting with 25% EtOAc/light petroleum, to give **59** (1.0 g, 92 %) as a tan oil, ¹H NMR δ 8.20 (ddd, *J* = 8.9, 2.2, 1.9 Hz, 2 H, H 3', H 5'), 7.53 (br d, *J* = 8.9 Hz, 2 H, H 2', H 6'), 7.23 (br d, *J* = 9.0 Hz, 2 H, H 2, H 6), 6.64 (ddd, *J* = 9.0, 3.4, 2.1 Hz, 2 H, H 3, H 5), 6.61 (br s, 1 H, OCONH), 5.92 (q, *J* = 6.6 Hz, 1 H, CHO), 3.67-3.71 (m, 4 H, 2 × CH₂N), 3.58-3.62 (m, 4 H, 2 × CH₂Cl), 1.59 (d, *J* = 6.6 Hz, 3 H, CH₃); ¹³C NMR δ 152.9, 149.3, 147.4, 142.9, 128.0, 126.6 (2), 123.8 (2), 121.4 (2), 112.7 (2), 71.9,

- 55 -

53.6 (2), 40.4 (2), 22.4; MS (FAB⁺) m/z 429 (M⁺, 5%), 427 (10), 425 (15); HRMS (FAB⁺) calc. for C₁₉H₂₁³⁵Cl₂N₃O₄ (M⁺) m/z 425.0909, found 425.0901; calc. for C₁₉H₂₁³⁵Cl³⁷ClN₃O₄ (M⁺) m/z 427.0880, found 427.0882; calc. for C₁₉H₂₁³⁷Cl₂N₃O₄ (M⁺) m/z 429.0850, found 429.0868.

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Example 3B. Preparation of 1-(4-nitrophenyl)ethyl 1-(chloromethyl)-3-[(5,6,7-trimethoxy-1*H*-indol-2-yl)carbonyl]-2,3-dihydro-1*H*-benzo[*e*]indol-5-ylcarbamate (60).

A solution of 1-(4-nitrophenyl)ethanol (56) (18 mg, 0.11 mmol) in DCM (2 mL) was added dropwise to a stirred solution of triphosgene (16 mg, 0.054 mmol) and pyridine (9 μL, 0.11 mmol) in DCM (2 mL) at 20 °C. The mixture was stirred at 20 °C for 2 h, the solvent evaporated and the residue dissolved in THF (5 mL). A solution of 1 [G. J. Atwell, W. R. Wilson, W. A. Denny, *Bioorg. Med. Chem. Lett.*, 1997, 7, 1483] (50 mg, 0.11 mmol) in THF (5 mL) was added and the solution stirred at 20 °C for 16 h. The mixture was partitioned between EtOAc (50 mL) and sat. aq. KHCO₃ solution, the organic fraction dried and the solvent evaporated. The residue was purified by chromatography, eluting with 25% EtOAc/light petroleum to give: (i) 60 (23 mg, 32%) as a tan solid mp (EtOAc/light petroleum) 175-178 °C; ¹H NMR δ 9.49 (s, 1 H, indole-NH), 8.88 (s, 1 H, OCONH), 8.18 (d, *J* = 7.6 Hz, 2 H, H 3", H 5"), 7.88 (d, *J* = 8.3 Hz, 1 H, H 6), 7.78 (d, *J* = 8.3 Hz, 1 H, H 9), 7.52-7.58 (m, 3 H, H 8, H 2", H 6"), 7.45 (dd, *J* = 7.8, 7.5 Hz, 1 H, H 7), 7.16 (br s, 1 H, H 4), 7.00 (d, *J* = 1.90 Hz, 1 H, H 3'), 6.87 (s, 1 H, H 4'), 6.00 (q, *J* = 6.6 Hz, 1 H, CHO), 4.80 (dd, *J* = 10.7, 1.2 Hz, 1 H, H 2), 4.65 (dd, *J* = 10.7, 8.8 Hz, 1 H, H 2), 4.11-4.17 (m, 1 H, CH₂Cl), 4.08 (s, 3 H, OCH₃), 3.93-3.97 (m, 4 H, OCH₃, CH₂Cl), 3.91 (s, 3 H, OCH₃), 3.45 (dt, *J* = 10.7, 3.3 Hz, 1 H, H 1), 1.65 (br d, *J* = 6.6 Hz, 3 H, CH₃); ¹³C NMR δ 160.3, 153.4, 150.2, 149.0, 147.5, 141.6, 140.6, 138.9, 133.8, 130.4, 129.7, 129.6, 127.4, 126.8 (2), 125.6, 125.0, 123.9, 123.8 (2), 123.6, 123.1, 122.3, 121.7, 106.5, 97.6, 72.6, 61.5, 61.1, 56.3, 54.9, 45.8, 43.4, 22.6; MS (FAB⁺) m/z 659 (MH⁺, 6%), 658 (6), 510 (1), 234 (10); HRMS calc. for C₃₄H₃₂³⁵ClN₄O₈ (MH⁺) m/z 659.1909, found 659.1881; calc for C₃₄H₃₂³⁷ClN₄O₈ (MH⁺) m/z 661.1879, found 661.1882; Anal. (C₃₄H₃₁ClN₄O₈) C, H, N: and (ii) starting material (1) (30 mg, 60 %).

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Example 3C. Preparation of 1-(2-methoxy-4-nitrophenyl)ethyl 4-[bis(2-chloroethyl)amino]phenylcarbamate (64).

- 56 -

2-Methoxy-4-nitrobenzaldehyde (61). PCC (0.76 g, 3.52 mmol) and 4Å molecular sieves (1.0 g) were added to a stirred solution of 2-methoxy-4-nitrobenzyl alcohol **29** (0.43 g, 2.35 mmol) in DCM (100 mL) and the suspension stirred at 20 °C for 1 h. The suspension was diluted with diethyl ether (150 mL) and the suspension filtered through Celite®, washed
5 with diethyl ether (2 × 20 mL). The combined organic fraction was evaporated and the residue purified by chromatography, eluting with 50% EtOAc/light petroleum, to give **61** (0.42 g, 98%) as white crystals, mp (EtOAc/light petroleum) 117-119 °C; ¹H NMR δ 10.52 (s, 1 H, CHO), 7.98 (br d, *J* = 8.2 Hz, 1 H, H 5), 7.85-7.89 (m, 2 H, H 3, H 6), 4.07 (s, 3 H, OCH₃); ¹³C NMR δ 188.2 (CHO), 161.8 (C 2), 152.2 (C 4), 129.5 (C 6), 128.6 (C 1), 115.6
10 (C 5), 107.2 (C 5), 56.4 (OCH₃); Anal. (C₈H₇NO₄) C, H, N.

1-(2-Methoxy-4-nitrophenyl)ethanol (62). A solution of MeMgBr (3 M in diethyl ether: 3.64 mL, 10.9 mmol) was added dropwise to a stirred solution of **61** (1.80 g, 9.93 mmol) in THF (100 mL) at -78 °C and the solution stirred at -78 °C for 20 min. The solution was
15 quenched with sat. aq NH₄Cl solution (5 mL) and allowed to warm to 20 °C. The solvent was evaporated and the residue purified by chromatography, eluting with 20% EtOAc/light petroleum, to give (i) starting material (0.38 g, 21%) and (ii) **62** (0.88 g, 45%) as a white solid, mp (EtOAc/light petroleum) 63-65 °C; ¹H NMR δ 7.86 (dd, *J* = 8.4, 2.1 Hz, 1 H, H 5), 7.70 (d, *J* = 2.1 Hz, 1 H, H 3), 7.58 (d, *J* = 8.4 Hz, 1 H, H 6), 5.19 (dq, *J* = 6.4, 4.7 Hz, 1
20 H, CHOCO), 3.96 (s, 3 H, OCH₃), 2.34 (d, *J* = 4.7 Hz, 1 H, OH), 1.48 (d, *J* = 6.4 Hz, 3 H, CH₃); ¹³C NMR δ 156.4 (C 2), 148.0 (C 4), 141.3 (C 1), 126.3 (C 6), 116.2 (C 5), 105.3 (C 3), 65.5 (CHOCO), 55.9 (OCH₃), 23.1 (CH₃); Anal. (C₉H₁₁NO₄) C, H, N.

1-(2-Methoxy-4-nitrophenyl)ethyl 4-[bis(2-hydroxyethyl)amino]phenylcarbamate (63). Pyridine (178 µL, 2.20 mmol) was added dropwise to a stirred solution of ethanol **62** (430 mg, 2.20 mmol) and triphosgene (229 mg, 0.77 mmol) in THF (50 mL) at 5 °C and the suspension stirred at 5 °C for 1 h. A solution of *N*¹,*N*¹-bis(2-hydroxyethyl)-1,4-benzenediamine **57** (476 mg, 2.42 mmol) with Pd/C under H₂ (60 psi) in EtOH) in THF (10 mL) and DMF (10 mL) was added and the mixture stirred at 20 °C for 10 days. The solvent
30 was evaporated and the residue purified by chromatography, eluting with EtOAc to give **63** (860 mg, 93%) as a tan foam, ¹H NMR δ 9.42 (br s, 1 H, OCONH), 7.91 (dd, *J* = 8.4, 2.1 Hz, 1 H, H 5), 7.79 (d, *J* = 2.1 Hz, 1 H, H 3), 7.60 (d, *J* = 8.4 Hz, 1 H, H 6), 7.18 (d, *J* = 9.0

Hz, 2 H, H 2', H 6'), 6.59 (d, $J = 9.0$ Hz, 2 H, H 3', H 5'), 6.00 (q, $J = 6.5$ Hz, 1 H, CHOCO), 4.69 (br s, 2 H, 2 \times OH), 3.98 (s, 3 H, OCH₃), 3.48-3.53 (m, 4 H, 2 \times CH₂O), 3.32-3.37 (m, 4 H, 2 \times CH₂N), 1.46 (d, $J = 6.5$ Hz, 3 H, CH₃); ¹³C NMR δ 155.7 (C 2), 152.6 (OCONH), 147.4 (C 4), 144.0 (C 4'), 138.7 (C 1'), 127.3 (C 1), 125.7 (C 6), 120.1 (C 2', C 6'), 115.8 (C 5), 111.4 (C 3', C 5'), 105.7 (C 3), 66.0 (CHOCO), 59.7 (2 \times CH₂O), 58.2 (OCH₃), 53.4 (2 \times CH₂N), 20.8 (CH₃); MS (FAB⁺) m/z 420 (MH⁺, 30%); HRMS (FAB⁺) calc. for C₂₀H₂₆N₃O₇ (MH⁺) m/z 420.1771, found 420.1761.

1-(2-Methoxy-4-nitrophenyl)ethyl 4-[bis(2-chloroethyl)amino]phenylcarbamate (64).

10 Methanesulphonyl chloride (404 μ L, 5.2 mmol) was added dropwise to a stirred solution of **63** (0.73 g, 1.74 mmol) in pyridine (20 mL) at 20 °C and the solution stirred for 1 h. The solvent was evaporated and the residue partitioned between DCM/water (100 mL). The aqueous fraction was extracted with DCM (2 \times 50 mL) and the combined organic fraction washed with brine (50 mL), dried and the solvent evaporated. The residue was dissolved in
 15 DMF (20 mL), LiCl (0.44 g, 10.4 mmol) added, and the mixture stirred at 80 °C for 3 h. The solvent was evaporated and the residue partitioned between EtOAc/water (200 mL). The aqueous fraction was extracted with EtOAc (2 \times 50 mL). The combined organic fraction was washed with brine (50 mL), dried, and the solvent evaporated. The residue was purified by chromatography, eluting with 20% EtOAc/light petroleum, to give **64** (0.53 g,
 20 67 %) as a tan foam, ¹H NMR δ 7.84 (dd, $J = 8.4, 1.9$ Hz, 1 H, H 5), 7.71 (d, $J = 1.9$ Hz, 1 H, H 3), 7.50 (d, $J = 8.4$ Hz, 1 H, H 6), 7.24 (br d, $J = 9.0$ Hz, 2 H, H 2', H 6'), 6.64 (d, $J = 9.0$ Hz, 2 H, H 3', H 5'), 6.62 (br s, 1 H, OCONH), 6.19 (q, $J = 6.5$ Hz, 1 H, CHOCO), 3.95 (s, 3 H, OCH₃), 3.69 (dd, $J = 7.0, 6.4$ Hz, 4 H, 2 \times CH₂N), 3.60 (dd, $J = 7.0, 6.4$ Hz, 4 H, 2 \times CH₂Cl), 1.52 (d, $J = 6.5$ Hz, 3 H, CH₃); ¹³C NMR δ 156.0 (C 2), 152.8 (OCONH), 148.1
 25 (C 4), 142.9 (C 4'), 138.5 (C 1'), 128.3 (C 1), 125.8 (C 6), 121.3 (C 2', C 6'), 116.0 (C 5), 112.8 (C 3', C 5'), 105.5 (C 3), 67.6 (CHOCO), 56.0 (OCH₃), 53.7 (2 \times CH₂N), 40.5 (2 \times CH₂Cl), 21.2 (CH₃); MS m/z 459 (M⁺, 2%), 457 (M⁺, 12), 455 (M⁺, 16), 276 (20), 231 (100); HRMS calc. for C₂₀H₂₃³⁵Cl₂N₃O₅ (M⁺) m/z 455.1015, found 455.1017; calc. for C₂₀H₂₃³⁵Cl³⁷ClN₃O₅ (M⁺) m/z 457.0985, found 457.0990; calc. for C₂₀H₂₃³⁷Cl₂N₃O₅ (M⁺) m/z
 30 459.0956, found 459.0972.

Example 4A. Preparation of 2-(2-hydroxyethoxy)-4-nitrobenzyl 1-(chloromethyl)-3-

- 58 -

[(5,6,7-trimethoxy-1*H*-indol-2-yl)carbonyl]-2,3-dihydro-1*H*-benzo[*e*]indol-5-ylcarbamate (70).

2-Bromoethyl *tert*-butyl(dimethyl)silyl ether (65). TBDMS triflate (5.0 mL, 21.8 mmol) was added dropwise to a stirred solution of bromoethanol (1.40 mL, 19.8 mmol) and
 5 pyridine (2.4 mL, 29.7 mmol) in DCM (50 mL) at 5 °C and the solution stirred at 20 °C for 16 h. MeOH (5 mL) was carefully added, the solution stirred for 5 min and poured into sat. aq. KHCO₃ (150 mL). The mixture was extracted with DCM (3 × 80 mL), the combined organic fraction dried, and the solvent evaporated. Chromatography of the residue, eluting with 10% EtOAc/light petroleum, gave 65 (3.95 g, 83%) as a colourless oil, ¹H NMR δ
 10 3.88 (t, *J* = 6.6 Hz, 2 H, CH₂O), 3.40 (t, *J* = 6.6 Hz, 2 H, CH₂Br), 0.90 (s, 9 H, SiC(CH₃)₃), 0.08 (s, 6 H, Si(CH₃)₂); ¹³C NMR δ 63.5 (CH₂O), 33.3 (CH₂Br), 25.8 (SiC(CH₃)₃), 18.3 (SiC(CH₃)₃), -5.3 (Si(CH₃)₂); MS (CI, NH₃) *m/z* 241 (MH⁺, 1%), 239 (MH⁺, 1%), 225 (2), 223 (2), 183 (55), 181 (55), 139 (100); HRMS (CI, NH₃) calc for C₈H₂₀⁷⁹BrOSi (MH⁺) *m/z* 239.0467; found 239.0460; calc for C₈H₂₀⁸¹BrOSi (MH⁺) *m/z* 241.0446; found 241.0450.

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Methyl 2-(2-{{*tert*-butyl(dimethyl)silyl}oxy}ethoxy)-4-nitrobenzoate (67). A mixture of methyl 2-hydroxy-4-nitrobenzoate (66) (0.55 g, 2.79 mmol) and K₂CO₃ (0.58 g, 4.19 mmol) in DMF (15 mL) was stirred at 20 °C for 30 min. A solution of 65 (1.00 g, 4.19 mmol) in DMF (5 mL) was added and the mixture was stirred at 100 °C for 4 h. The
 20 solvent was evaporated and the residue partitioned between EtOAc (100 mL) and water (100 mL). The organic fraction was washed with water (2 × 50 mL), brine (50 mL), dried, and the solvent evaporated. The residue was purified by chromatography, eluting with 20% EtOAc/light petroleum, to give 67 (0.76 g, 77%) as a white solid, mp (EtOAc/light petroleum) 47-48 °C; ¹H NMR δ 7.89 (d, *J* = 1.3 Hz, 1 H, H 3), 7.81-7.85 (m, 2 H, H 5, H
 25 6), 4.24 (t, *J* = 5.0 Hz, 2 H, CH₂O), 4.03 (t, *J* = 5.0 Hz, 2 H, CH₂O), 3.92 (s, 3 H, OCH₃), 0.88 (s, 9 H, SiC(CH₃)₃), 0.08 (s, 6 H, Si(CH₃)₂); ¹³C NMR (CDCl₃) δ 165.5 (CO₂), 158.6 (C 2), 150.4 (C 4), 131.8 (C 6), 126.7 (C 1), 115.0 (C 5), 108.3 (C 3), 71.1 (CH₂O), 61.7 (CH₂O), 52.5 (OCH₃), 25.7 (SiC(CH₃)₃), 18.3 (SiC(CH₃)₃), -5.5 (Si(CH₃)₂); Anal. (C₁₆H₂₅NO₆Si) C, H, N.

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[2-(2-{{*tert*-Butyl(dimethyl)silyl}oxy}ethoxy)-4-nitrophenyl]methanol (68). DIBALH (1M in DCM, 6.7 mL, 6.7 mmol) was added dropwise to a stirred solution of ester 67 (0.72

g, 2.02 mmol) in THF (50 mL) at 5 °C and the solution stirred at 5 °C for 1 h. The solution was poured into a solution of potassium sodium tartrate (1 M, 50 mL) and stirred vigorously for 30 min. The mixture was extracted with EtOAc (3 × 50 mL), the combined organic fraction washed with water (50 mL), brine (50 mL), dried and the solvent was
 5 evaporated. The residue was purified by chromatography, eluting with 20% EtOAc/light petroleum, to give **68** (0.64 g, 97%) as a white solid, mp (EtOAc/light petroleum) 89-90 °C; ¹H NMR δ 7.85 (dd, *J* = 8.2, 2.1 Hz, 1 H, H 5), 7.74 (d, *J* = 2.1 Hz, 1 H, H 3), 7.46 (d, *J* = 8.2 Hz, 1 H, H 6), 4.75 (s, 2 H, CH₂O), 4.21 (dd, *J* = 4.9, 4.4 Hz, 2 H, CH₂O), 4.01 dd, *J* = 4.9, 4.4 Hz, 2 H, CH₂O), 2.84 (br s, 1 H, OH), 0.90 (s, 9 H, SiC(CH₃)₃), 0.06 (s, 6 H, Si(CH₃)₂); ¹³C NMR δ 157.0 (C 2), 148.3 (C 4), 137.1 (C 1), 128.4 (C 6), 116.3 (C 5), 106.8 (C 3), 70.5 (CH₂O), 61.6 (CH₂O), 61.3 (CH₂O), 25.8 (SiC(CH₃)₃), 18.3 (SiC(CH₃)₃), -5.4 (Si(CH₃)₂); Anal. (C₁₅H₂₅NO₅Si) C, H, N.

2-(2-([*tert*-Butyl(dimethyl)silyl]oxy)ethoxy)-4-nitrobenzyl 1-(chloromethyl)-3-[(5,6,7-trimethoxy-1*H*-indol-2-yl)carbonyl]-2,3-dihydro-1*H*-benzo[*e*]indol-5-ylcarbamate
 15 **(69)**. A solution of triphosgene (24 mg, 80 μmol) in DCM (2 mL) was added dropwise to a stirred solution of amine **1** [G. J. Atwell, W. R. Wilson, W. A. Denny, *Bioorg. Med. Chem. Lett.*, **1997**, *7*, 1483] (107 mg, 230 μmol) and Et₃N (64 μL, 460 μmol) in DCM (20 mL) and stirred at 20 °C for 2 h. A solution of [2-(2-([*tert*-butyl(dimethyl)silyl]oxy)ethoxy)-4-nitrophenyl]methanol (**68**) (83 mg, 253 μmol) in DCM (5 mL) was added, followed by
 20 *n*Bu₂Sn(OAc)₂ (2 drops) and the solution stirred at 20 °C for 24 h. The solvent was evaporated and the residue purified by chromatography, eluting with a gradient (0-20%) EtOAc/DCM, to give **69** (177 mg, 94%) as a white solid, mp (EtOAc/light petroleum) 182-185 °C; ¹H NMR δ 9.45 (s, 1 H, indole-NH), 8.93 (s, 1 H, OCONH), 7.92 (d, *J* = 8.5 Hz, 1 H, H 6), 7.76-7.83 (m, 3 H, H 9, H 3'', H 5''), 7.53-7.60 (m, 2 H, H 8, H 6''), 7.47 (ddd, *J* = 8.5, 7.1, 0.8 Hz, 1 H, H 7), 7.13 (br s, 1 H, H 4), 7.01 (d, *J* = 2.2 Hz, 1 H, H 3'), 6.88 (s, 1 H, H 4'), 5.39 (s, 2 H, CH₂O), 4.81 (dd, *J* = 10.7, 1.8 Hz, 1 H, H 2), 4.67 (dd, *J* = 10.7, 8.7 Hz, 1 H, H 2), 4.21 (br dd, *J* = 5.0, 4.8 Hz, 2 H, CH₂O), 4.15-4.18 (m, 1 H, H 1), 4.09 (s, 3 H, OCH₃), 4.02 (br d, *J* = 5.0, 4.8 Hz, 2 H, CH₂O), 3.97 (d, *J* = 11.5, 3.1 Hz, 1 H, CH₂Cl),
 30 3.95 (s, 3 H, OCH₃), 3.92 (s, 3 H, OCH₃), 3.48 (dd, *J* = 11.5, 10.9 Hz, 1 H, CH₂Cl), 0.90 (s, 9 H, SiC(CH₃)₃), 0.10 (s, 6 H, Si(CH₃)₂); ¹³C NMR δ 160.3 (CO), 156.7 (C 2''), 154.0 (OCONH), 150.2 (C 5'), 148.5 (C 4''), 141.7 (C 3a), 140.6 (C 6'), 138.9 (C 7'), 133.8 (C

- 5), 132.3 (C 1''), 129.7 (C 2'), 129.6 (C 9a), 128.7 (C 6''), 127.5 (C 8), 125.6 (C 7a'), 125.0 (C 7), 123.6 (C 3a'), 123.1 (C 9), 122.4 (C 6, C 9b), 121.8 (C 5a), 115.8 (C 5''), 112.8 (C 4), 106.5 (C 3', C 3''), 97.7 (C 4'), 70.7 (CH₂O), 61.8 (CH₂O), 61.7 (CH₂O), 61.5 (OCH₃), 61.1 (OCH₃), 56.3 (OCH₃), 54.9 (C 2), 45.8 (CH₂Cl), 43.5 (C 1), 25.8 (SiC(CH₃)₃), 18.3 (SiC(CH₃)₃), -5.4 (Si(CH₃)₂); MS (FAB⁺) *m/z* 819 (MH⁺, 25%), 821 (MH⁺, 12); HRMS (FAB⁺) calcd for C₄₁H₄₈³⁵ClN₄O₁₀Si (MH⁺) *m/z* 819.2828, found 819.2804; calc. for C₄₁H₄₈³⁷ClN₄O₁₀Si (MH⁺) *m/z* 821.2799, found 821.2803; Anal. (C₄₁H₄₇ClN₄O₁₀Si) C, H, N.
- 10 **2-(2-Hydroxyethoxy)-4-nitrobenzyl 1-(chloromethyl)-3-[(5,6,7-trimethoxy-1*H*-indol-2-yl)carbonyl]-2,3-dihydro-1*H*-benzo[*e*]indol-5-ylcarbamate (70).** 1 M HCl (0.4 mL, 400 μmol) was added to a stirred solution of silyl ether **69** (157 mg, 192 μmol) in MeOH (5 mL) and the solution stirred at 20 °C for 1 h. The solvent was evaporated and the residue partitioned between EtOAc (50 mL) and water (50 mL). The organic fraction was washed
- 15 with water (50 mL), brine (25 mL), dried, and the solvent evaporated. The residue was purified by chromatography, eluting with a gradient (20-50%) of EtOAc/light petroleum, to give **70** (119 mg, 88%) as a hygroscopic white solid, ¹H NMR δ 9.72 (s, 1 H, indole-NH), 8.80 (s, 1 H, OCONH), 7.86 (d, *J* = 8.5 Hz, 1 H, H 6), 7.79 (br d, *J* = 8.1 Hz, 1 H, H 5''), 7.67-7.73 (m, 2 H, H 9, H 3''), 7.47-7.53 (m, 3 H, H 4, H 8, H 6''), 7.37 (ddd, *J* = 8.5, 7.1,
- 20 0.8 Hz, 1 H, H 7), 6.97 (d, *J* = 2.2 Hz, 1 H, H 3'), 6.87 (s, 1 H, H 4'), 5.40 (s, 2 H, CH₂O), 4.73 (dd, *J* = 10.7, 1.6 Hz, 1 H, H 2), 4.59 (dd, *J* = 10.7, 8.7 Hz, 1 H, H 2), 4.21 (br dd, *J* = 4.6, 4.0 Hz, 2 H, CH₂O), 4.07-4.11 (m, 4 H, H 1, OCH₃), 4.00-4.04 (m, 2 H, CH₂O), 3.95 (s, 3 H, OCH₃), 3.92 (s, 3 H, OCH₃), 3.85 (d, *J* = 11.3, 3.0 Hz, 1 H, CH₂Cl), 3.39 (br s, 1 H, OH), 3.28 (dd, *J* = 11.3, 10.9 Hz, 1 H, CH₂Cl); ¹³C NMR δ 160.5 (CO), 157.2 (C 2''), 154.4 (OCONH), 150.2 (C 5'), 148.9 (C 4''), 141.4 (C 3a), 140.6 (C 6'), 138.9 (C 7'), 133.8 (C 5), 131.9 (C 1''), 130.3 (C 6''), 129.6 (C 2', C 9a), 127.4 (C 8), 125.8 (C 7a'), 125.0 (C 7), 123.6 (C 3a'), 123.0 (C 9), 122.5 (C 6, C 9b), 121.9 (C 5a), 115.9 (C 5''), 112.8 (C 4), 106.7 (C 3'), 106.6 (C 3''), 97.7 (C 4'), 70.7 (CH₂O), 62.0 (CH₂O), 61.5 (OCH₃), 61.1 (OCH₃), 60.9 (CH₂O), 56.3 (OCH₃), 55.1 (C 2), 45.6 (CH₂Cl), 43.3 (C 1); MS (FAB⁺) *m/z*
- 25 707 (MH⁺, 5%), 705 (MH⁺, 14); HRMS (FAB⁺) calcd for C₃₅H₃₄³⁵ClN₄O₁₀ (MH⁺) *m/z* 705.1964, found 705.1919; calc. for C₃₅H₃₄³⁷ClN₄O₁₀ (MH⁺) *m/z* 707.1934, found 707.1931; Anal. (C₃₅H₃₃ClN₄O₁₀) C, H, N.
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Example 4B. Preparation of 2-(2-methoxyethoxy)-4-nitrobenzyl 1-(chloromethyl)-3-[(5,6,7-trimethoxy-1*H*-indol-2-yl)carbonyl]-2,3-dihydro-1*H*-benzo[*e*]indol-5-ylcarbamate (73).

- 5 **Methyl 2-(2-methoxyethoxy)-4-nitrobenzoate (71).** A mixture of methyl 2-hydroxy-4-nitrobenzoate (66) (1.0 g, 5.07 mmol) and K_2CO_3 (1.05 g, 7.61 mmol) in DMF (25 mL) was stirred at 20 °C for 30 min. A solution of 2-bromoethyl methyl ether (0.72 mL, 7.61 mmol) in DMF (3 mL) was added and the mixture was stirred at 100 °C for 4 h. The solvent was evaporated and the residue partitioned between EtOAc (100 mL) and water (100 mL). The
10 organic fraction was washed with water (2 × 50 mL), brine (50 mL), dried, and the solvent evaporated. The residue was purified by chromatography, eluting with 30% EtOAc/light petroleum, to give **71** (1.27 g, 98%) as a white solid, mp (EtOAc/light petroleum) 45-46 °C; 1H NMR δ 7.90 (d, J = 8.3 Hz, 1 H, H 6), 7.82-7.86 (m, 2 H, H 3, H 5), 4.28-4.30 (m, 2 H, CH_2O), 3.93 (s, 3 H, OCH_3), 3.82-3.87 (m, 2 H, CH_2O), 3.48 (s, 3 H, OCH_3); ^{13}C NMR δ
15 165.1 (CO_2), 158.6 (C 2), 150.6 (C 4), 132.1 (C 6), 126.4 (C 1), 115.2 (C 5), 108.4 (C 3), 70.5 (CH_2O), 69.4 (CH_2O), 59.4 (OCH_3), 52.5 (OCH_3); Anal. ($C_{11}H_{13}NO_6$) C, H, N.

- 2-[2-(Methoxy)ethoxy]-4-nitrobenzyl alcohol (72).** DIBALH (1 M in DCM, 16.4 mL, 16.4 mmol) was added dropwise to a stirred solution of ester **71** (1.27 g, 4.97 mmol) in
20 THF (100 mL) at 5 °C and the solution stirred at 5 °C for 1 h. The solution was poured into a solution of potassium sodium tartrate (1 M, 100 mL) and stirred vigorously for 30 min. The mixture was extracted with EtOAc (2 × 100 mL), the combined organic fraction washed with water (50 mL), brine (50 mL), dried and the solvent was evaporated. The residue was purified by chromatography, eluting with a gradient (30-50%) of EtOAc/light
25 petroleum, to give **72** (1.03 g, 91%) as a white solid, mp (EtOAc/light petroleum) 89-90.5 °C; 1H NMR δ 7.86 (dd, J = 8.2, 2.1 Hz, 1 H, H 5), 7.72 (d, J = 2.1 Hz, 1 H, H 3), 7.47 (d, J = 8.2 Hz, 1 H, H 6), 4.74 (br s, 2 H, CH_2O), 4.26-4.29 (m, 2 H, CH_2O), 3.78-3.80 (m, 2 H, CH_2O), 3.45 (s, 3 H, OCH_3), 3.10 (br s, 1 H, OH); ^{13}C NMR δ 156.8 (C 2), 148.2 (C 4), 137.5 (C 1), 128.6 (C 6), 116.5 (C 5), 107.0 (C 3), 70.5 (CH_2O), 68.4 (CH_2O), 61.1 (CH_2O),
30 59.1 (OCH_3); Anal. ($C_{10}H_{13}NO_5$) C, H, N.

2-(2-Methoxyethoxy)-4-nitrobenzyl 1-(chloromethyl)-3-[(5,6,7-trimethoxy-1*H*-indol-2-

yl)carbonyl]-2,3-dihydro-1H-benzo[e]indol-5-ylcarbamate (73). A solution of triphosgene (12 mg, 40 μ mol) in DCM (2 mL) was added dropwise to a stirred solution of amine 1 [G. J. Atwell, W. R. Wilson, W. A. Denny, *Bioorg. Med. Chem. Lett.*, **1997**, *7*, 1483] (53 mg, 114 μ mol) and Et₃N (32 μ L, 228 μ mol) in DCM (10 mL) and stirred at 20 °C for 2 h. A solution of 2-[2-(methoxy)ethoxy]-4-nitrobenzyl alcohol 72 (28 mg, 125 μ mol) in DCM (2 mL) was added, followed by nBu₂Sn(OAc)₂ (2 drops) and the solution stirred at 20 °C for 24 h. The solvent was evaporated and the residue purified by chromatography, eluting with 20% EtOAc/DCM, to give 73 (75 mg, 91%) as a tan gum, ¹H NMR δ 9.49 (s, 1 H, indole-NH), 8.19 (s, 1 H, OCONH), 7.92 (d, *J* = 8.5 Hz, 1 H, H 6), 7.80-7.82 (m, 1 H, H 5"), 7.78 (d, *J* = 8.3 Hz, 1 H, H 9), 7.71 (d, *J* = 1.8 Hz, 1 H, H 3"), 7.56 (ddd, *J* = 8.3, 7.1, 0.8 Hz, 1 H, H 8), 7.49-7.54 (m, 1 H, H 6"), 7.45 (ddd, *J* = 8.5, 7.1, 0.8 Hz, 1 H, H 7), 7.27 (br s, 1 H, H 4), 7.00 (d, *J* = 2.3 Hz, 1 H, H 3'), 6.87 (s, 1 H, H 4'), 5.39 (s, 2 H, CH₂O), 4.79 (dd, *J* = 10.7, 1.7 Hz, 1 H, H 2), 4.66 (dd, *J* = 10.7, 8.7 Hz, 1 H, H 2), 4.23 (dd, *J* = 4.6, 4.4 Hz, 2 H, CH₂O), 4.15-4.20 (m, 1 H, H 1), 4.08 (s, 3 H, OCH₃), 3.94-3.98 (m, 4 H, OCH₃, CH₂Cl), 3.91 (s, 3 H, OCH₃), 3.80 (dd, *J* = 4.6, 4.4 Hz, 2 H, CH₂O), 3.47 (d, *J* = 10.9 Hz, 1 H, CH₂Cl), 3.44 (s, 3 H, OCH₃); ¹³C NMR δ 160.3 (CO), 156.5 (C 2"), 154.0 (OCONH), 150.2 (C 5'), 148.5 (C 4"), 141.7 (C 3a), 140.6 (C 6'), 138.9 (C 7'), 133.9 (C 5), 132.4 (C 1"), 129.7 (C 2'), 129.6 (C 9a), 128.7 (C 6"), 127.5 (C 8), 125.6 (C 7a'), 125.0 (C 7), 123.6 (C 3a'), 123.1 (C 9), 122.5 (C 6, C 9b), 121.8 (C 5a), 116.0 (C 5"), 112.7 (C 4), 106.5 (C 3'), 106.3 (C 3"), 97.7 (C 4'), 70.7 (CH₂O), 68.5 (CH₂O), 61.9 (CH₂O), 61.5 (OCH₃), 61.1 (OCH₃), 59.3 (OCH₃), 56.3 (OCH₃), 54.9 (C 2), 45.9 (CH₂Cl), 43.1 (C 1); MS (FAB⁺) *m/z* 721 (MH⁺, 1.5%), 719 (MH⁺, 3.5); HRMS (FAB⁺) calc. for C₃₆H₃₆³⁷ClN₄O₁₀ (MH⁺) *m/z* 721.2091, found 721.2131; calc. for C₃₆H₃₆³⁵ClN₄O₁₀ (MH⁺) *m/z* 719.2120, found 719.2133; Anal. (C₃₆H₃₅ClN₄O₁₀) C, H, N.

Example 4C. Preparation of 1-[2-(3-hydroxypropoxy)-4-nitrophenyl]ethyl 4-[bis(2-chloroethyl)amino]phenylcarbamate (78).

Methyl 4-nitro-2-[3-(tetrahydro-2H-pyran-2-yloxy)propoxy]benzoate (74). A mixture of methyl 4-nitrosalicylate (66) (2.3 g, 11.7 mmol) and K₂CO₃ (2.42 g, 17.5 mmol) in DMF (25 mL) was stirred at 20 °C for 20 min. A solution of 3-iodopropyl tetrahydropyranyl ether (4.7 g, 17.5 mmol) in DMF (5 mL) was added and the mixture stirred at 100 °C for 2 h. The mixture was poured into water, extracted with EtOAc (3 \times 100 mL), the combined

organic extracts washed with water (2 × 50 mL), brine (50 mL), dried and the solvent evaporated. The residue was purified by chromatography, eluting with 20% EtOAc/light petroleum, to give **74** (3.66 g, 92 %) as a colourless oil, ¹H NMR δ 7.89 (d, *J* = 8.5 Hz, 1 H, H 6), 7.80-7.84 (m, 2 H, H 3, H 5), 4.60-4.62 (m, 1 H, OCHO), 4.27 (t, *J* = 6.2 Hz, 2 H, CH₂O), 3.95-4.00 (m, 1 H CH₂O), 3.94 (s, 3 H, OCH₃), 3.79-3.86 (m, 1 H, CH₂O), 3.59-3.66 (m, 1 H, CH₂O), 3.47-3.52 (m, 1 H, CH₂O), 2.13-2.17 (m, 2 H, CH₂), 1.78-1.84 (m, 1 H, CH₂), 1.68-1.75 (m, 1 H, CH₂), 1.47-1.62 (m, 4 H, 2 CH₂); ¹³C NMR δ 164.5, 158.6, 150.7, 132.0, 126.2, 114.8, 107.9, 99.0, 66.5, 63.4, 62.4, 52.5, 30.6, 29.3, 25.4, 19.6; MS *m/z* 339 (M⁺, 2%), 322 (12), 239 (20), 222(40), 85 (100); HRMS calc. for C₁₆H₂₁NO₇ (M⁺) *m/z* 339.1318, found 339.1317.

{4-Nitro-2-[3-(tetrahydro-2*H*-pyran-2-yloxy)propoxy]phenyl}methanol (75). DIBALH (1 M in DCM, 34 mL, 34 mmol) was added dropwise to a solution of **74** (3.46 g, 10.2 mmol) in THF (100 mL) at 5 °C and the solution stirred at 5 °C for 1 h. The solution was poured into a solution of sodium potassium tartrate (1 M, 100 mL) and stirred for 30 min. The mixture was extracted with EtOAc (3 × 100 mL), the combined organic fraction washed with water (100 mL), brine (50 mL), dried, and the solvent evaporated. The residue was purified by chromatography, eluting with 50%EtOAc/light petroleum, to give **75** (3.11 g, 98 %) as a pale yellow solid, mp (EtOAc/light petroleum) 64-65.5 °C; ¹H NMR δ 7.84 (dd, *J* = 8.2, 2.1 Hz, 1 H, H 5), 7.72 (d, *J* = 2.1 Hz, 1 H, H 3), 7.50 (d, *J* = 8.2 Hz, 1 H, H 6), 4.74 (dd, *J* = 14.8, 4.2 Hz, 2 H, CH₂O), 4.58-4.61 (m, 1 H, OCHO), 4.24 (t, *J* = 6.1 Hz, 2 H, CH₂O), 3.96 (dt, *J* = 10.0, 5.8 Hz, 1 H, CH₂O), 3.80-3.86 (m, 1 H, CH₂O), 3.62 (dt, 10.0, 5.8 Hz, 1 H, CH₂O), 3.46-3.51 (m, 1 H, CH₂), 2.30 (br s, 1 H, OH), 2.08-2.11 (m, 2 H, CH₂), 1.79-1.85 (m, 1 H, CH₂), 1.69-1.77 (m, 1 H, CH₂), 1.48-1.62 (m, 4 H, 2 × CH₂); ¹³C NMR δ 156.5, 148.2, 136.8, 128.1, 115.9, 105.8, 99.3, 65.9, 63.9, 62.8, 60.8, 30.6, 29.3, 25.3, 19.7; MS (CI, NH₃) *m/z* 312 (MH⁺, 0.5%), 294 (1), 245 (15), 227(30), 85 (100); HRMS (CI, NH₃) calc. for C₁₅H₂₂NO₆ (MH⁺) *m/z* 312.1447, found 312.1438. Anal. (C₁₅H₂₁NO₆) C, H, N.

4-Nitro-2-[3-(tetrahydro-2*H*-pyran-2-yloxy)propoxy]benzyl 4-[bis(2-hydroxyethyl)amino]phenylcarbamate (76). Pyridine (135 μL, 1.67 mmol) was added dropwise to a stirred solution of alcohol **75** (521 mg, 1.67 mmol) and triphosgene (174 mg,

- 64 -

0.59 mmol) in THF (20 mL) at 5 °C and the suspension stirred at 5 °C for 1 h. A solution of *N,N'*-bis(2-hydroxyethyl)-1,4-benzenediamine **57** [prepared by catalytic hydrogenation of *N,N*-bis-(2-hydroxyethyl) 4-nitroaniline (360 mg, 1.84 mmol) with Pd/C under H₂ (60 psi) in EtOH] in THF (10 mL) and DMF (10 mL) was added and the mixture stirred at 20 °C for 16 h. The solvent was evaporated and the residue partitioned between EtOAc/water (100 mL). The aqueous fraction was extracted with EtOAc (2 × 50 mL) and the combined organic fraction washed with brine (50 mL), dried and the solvent evaporated. The residue was purified by chromatography, eluting with 80-100% EtOAc/light petroleum to give **76** (220 mg, 25 %) as a colourless oil, ¹H NMR [(CD₃)₂SO] δ 9.42 (s, 1 H, OCONH), 7.88 (dd, *J* = 8.3, 2.0 Hz, 1 H, H 5'), 7.79 (d, *J* = 2.0 Hz, 1 H, H 3'), 7.61 (br d, *J* = 8.3 Hz, 1 H, H 6'), 7.72 (br d, *J* = 9.1 Hz, 2 H, H 2, H 6), 6.61 (d, *J* = 9.1 Hz, 2 H, H 3, H 5), 5.19 (s, 2 H, CH₂O), 4.73 (t, *J* = 5.4 Hz, 1 H, CH₂O), 4.54-4.58 (m, 1 H, OCHO), 4.23 (t, *J* = 6.1 Hz, 2 H, CH₂O), 3.80 (dt, *J* = 9.9, 6.4 Hz, 1 H, CH₂O), 3.67-3.71 (m, 1 H, CH₂O), 3.55 (t, *J* = 5.4 Hz, 1 H, CH₂O), 3.50 (t, *J* = 6.0 Hz, 4 H, 2 × CH₂O), 3.32-3.35 (m, 4 H, 2 × CH₂N), 1.99-2.06 (m, 2 H, CH₂), 1.67-1.73 (m, 1 H, CH₂), 1.56-1.62 (m, 1 H, CH₂), 1.40-1.49 (m, 4 H, 2 × CH₂); ¹³C NMR [(CD₃)₂SO] δ 156.2, 153.2, 148.1, 144.1, 132.9, 128.6, 127.4, 120.2 (2), 115.4, 111.4 (2), 106.0, 98.0, 65.7, 63.0, 61.3, 60.1, 58.1 (2), 53.4 (2), 30.2, 28.8, 25.0, 19.1; MS (FAB⁺) *m/z* 533 (M⁺, 20 %); HRMS (FAB⁺) calc. for C₂₆H₃₅N₃O₉ (M⁺) *m/z* 533.2373, found 533.2365.

20

2-(3-Hydroxypropoxy)-4-nitrobenzyl 4-[bis(2-chloroethyl)amino]phenylcarbamate (78). Methanesulphonyl chloride (85 μL, 1.1 mmol) was added dropwise to a stirred solution of **76** (195 mg, 0.36 mmol) in pyridine (10 mL) at 20 °C and the solution stirred for 1 h. The solvent was evaporated and the residue partitioned between DCM/water (100 mL). The aqueous fraction was extracted with DCM (2 × 50 mL) and the combined organic fraction washed with brine (50 mL), dried and the solvent evaporated. The residue was dissolved in DMF (10 mL), LiCl (93 mg, 2.2 mmol) added, and the mixture stirred at 80 °C for 3 h. The solvent was evaporated and the residue partitioned between EtOAc (100 mL) and water (100 mL). The aqueous fraction was extracted with EtOAc (2 × 50 mL). The combined organic fraction was washed with brine (50 mL), dried, and the solvent evaporated. The residue was purified by chromatography, eluting with 25% EtOAc/light petroleum, to give (i) 4-nitro-2-[3-(tetrahydro-2*H*-pyran-2-yloxy)propoxy]benzyl 4-[bis(2-

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- 65 -

chloroethyl)amino]phenylcarbamate (77) (46 mg, 22 %) as a colourless oil, ^1H NMR δ 7.82 (dd, $J = 8.3, 2.1$ Hz, 1 H, H 5'), 7.73 (d, $J = 2.1$ Hz, 1 H, H 3'), 7.50 (br d, $J = 8.3$ Hz, 1 H, H 6'), 7.26-7.29 (m, 3 H, OCONH, H 2, H 6), 6.65 (ddd, $J = 9.1, 3.4, 1.9$ Hz, 2 H, H 3, H 5), 5.25 (s, 2 H, CH_2O), 4.67 (br s, 1 H, OCHO), 4.20-4.27 (m, 2 H, CH_2O), 4.01 (dt, $J =$
 5 9.7, 6.1 Hz, 1 H, CH_2O), 3.65-3.75 (m, 6 H, CH_2O , $2 \times \text{CH}_2\text{N}$), 3.58-3.63 (m, 4 H, $2 \times \text{CH}_2\text{Cl}$), 3.44-3.50 (m, 1 H, CH_2O), 2.11-2.15 (m, 2 H, CH_2), 1.66-1.72 (m, 1 H, CH_2), 1.55-1.62 (m, 1 H, CH_2), 1.40-1.50 (m, 4 H, $2 \times \text{CH}_2$); ^{13}C NMR δ 157.2, 153.6, 148.8, 142.6, 132.0, 129.8, 128.7, 121.3 (2), 115.5, 112.8 (2), 105.9, 98.4, 65.3, 63.0, 61.7 (2), 53.7 (2), 40.5 (2), 30.5, 29.3, 25.4, 19.0; MS (FAB $^+$) m/z 569 (M^+ , 3%); HRMS (FAB $^+$) calc for
 10 $\text{C}_{26}\text{H}_{33}^{35}\text{Cl}_2\text{N}_3\text{O}_7$ (M^+) m/z 569.1696, found 569.1689; calc. for $\text{C}_{26}\text{H}_{33}^{35}\text{Cl}^{37}\text{ClN}_3\text{O}_7$ (M^+) m/z 571.1666, found 569.1680; calc. for $\text{C}_{26}\text{H}_{33}^{37}\text{Cl}_2\text{N}_3\text{O}_7$ (M^+) m/z 573.1637, found 569.1654.

Further elution gave 78 (99 mg, 57 %) as a white powder, mp (DCM/pet. ether) 104-105
 $^\circ\text{C}$; ^1H δ 7.84 (dd, $J = 8.3, 2.0$ Hz, 1 H, H 5'), 7.74 (d, $J = 2.0$ Hz, 1 H, H 3'), 7.51 (br d, $J =$
 15 8.3 Hz, 1 H, H 6'), 7.24 (br d, $J = 9.0$ Hz, 2 H, H 2, H 6), 6.87 (br s, 1 H, OCONH), 6.64 (d, $J = 9.0$ Hz, 2 H, H 3, H 5), 5.27 (s, 2 H, CH_2O), 4.27 (t, $J = 5.8$ Hz, 2 H, CH_2O), 3.89 (t, $J = 5.7$ Hz, 2 H, CH_2O), 3.67-3.72 (m, 4 H, $2 \times \text{CH}_2\text{N}$), 3.58-3.64 (m, 4 H, $2 \times \text{CH}_2\text{Cl}$), 2.08-2.12 (m, 2 H, CH_2); ^{13}C NMR δ 156.8, 153.4, 148.7, 145.6, 132.0, 129.4, 128.2, 123.4 (2), 115.6, 112.7 (2), 106.1, 66.6, 61.5, 59.9, 53.6 (2), 40.5 (2), 31.6; Anal. ($\text{C}_{21}\text{H}_{25}\text{Cl}_2\text{N}_3\text{O}_6$) C,
 20 H, N, Cl.

Example 4D. Preparation of 2-(3-hydroxypropoxy)-4-nitrobenzyl 3-(chloromethyl)-1-[(5,6,7-trimethoxy-1*H*-indol-2-yl)carbonyl]-2,3-dihydro-1*H*-indol-6-ylcarbamate (80).
 Pyridine (35 μL , 0.44 mmol) was added dropwise to a stirred solution of alcohol 75 (45
 25 mg, 0.25 mmol) and triphosgene (45 mg, 0.15 mmol) in THF (10 mL) at 5 $^\circ\text{C}$ and the suspension stirred at 5 $^\circ\text{C}$ for 1 h. A solution of 3-(chloromethyl)-1-[(5,6,7-trimethoxy-1*H*-indol-2-yl)carbonyl]-2,3-dihydro-1*H*-indol-6-ylamine (33) [M. Tercel and W. A. Denny. *J. Chem. Soc. Perkin Trans. 1*, 1998, 509] (199 mg, 0.48 mmol) in THF (10 mL) was added and the mixture stirred at 20 $^\circ\text{C}$ for 16 h. The suspension was filtered and the solvent
 30 evaporated. The residue was purified by chromatography, eluting with 40% EtOAc/DCM, to give (i) 4-nitro-2-[3-(tetrahydro-2*H*-pyran-2-yloxy)propoxy]benzyl 3-(chloromethyl)-1-[(5,6,7-trimethoxy-1*H*-indol-2-yl)carbonyl]-2,3-dihydro-1*H*-indol-6-ylcarbamate 79 (116

mg, 35%) as an oil, ^1H NMR δ 9.54 (s, 1 H, NH), 8.24 (s, 1 H, H 7), 7.84 (s, 1 H, H 4), 7.77 (dd, $J = 8.3, 2.0$ Hz, 1 H, H 5''), 7.71 d, $J = 2.0$ Hz, 1 H, H 3''), 7.52 (br s, 1 H, OCONH), 7.48 (d, $J = 8.3$ Hz, 1 H, H 6''), 7.22 (d, $J = 8.3$ Hz, 1 H, H 4), 6.93 (d, $J = 2.2$ Hz, 1 H, H 3'), 6.85 (s, 1 H, H 4'), 5.27 (s, 2 H, CH_2O), 4.69 (br s, 1 H, OCHO), 4.62 (dd, $J = 10.6, 9.4$ Hz, 1 H, H 2), 4.45 (dd, $J = 10.6, 3.8$ Hz, 1 H, H 2), 4.20-4.24 (m, 2 H, CH_2O), 4.08 (s, 3 H, OCH_3), 3.94-3.98 (m, 1 H, CH_2O), 3.93 (s, 3 H, OCH_3), 3.91 (s, 3 H, OCH_3), 3.71-3.82 (m, 3 H, H 3, CH_2O , CH_2Cl), 3.49-3.61 (m, 3 H, CH_2Cl , CH_2O), 2.10-2.15 (m, 2 H, CH_2), 1.65-1.78 (m, 2 H, CH_2), 1.45-1.60 (m, 4 H, $2 \times \text{CH}_2$); ^{13}C NMR δ 160.3, 157.2, 153.3, 150.2, 148.8, 144.1, 140.5, 138.9, 138.8, 131.8, 129.6, 129.5, 125.9, 125.5, 124.5, 123.6, 115.5, 114.6, 108.7, 107.9, 105.9, 98.4, 97.6, 67.6, 65.4, 63.0, 61.8, 61.4, 61.1, 56.2, 54.7, 46.9, 43.2, 30.6, 29.2, 25.4, 19.0; MS (FAB $^+$) m/z 752 (M^+ , 8%), 669 (20), 234 (30); HRMS (FAB $^+$) calc. for $\text{C}_{37}\text{H}_{41}^{35}\text{ClN}_4\text{O}_{11}$ (M^+) m/z 752.2460, found 752.2455; calc. for $\text{C}_{37}\text{H}_{41}^{37}\text{ClN}_4\text{O}_{11}$ (M^+) m/z 754.2431, found 754.2424.

15 Further elution gave **80** (44 mg, 15%) as a tan solid, mp (EtOAc/light petroleum) 166-168 °C; ^1H NMR δ 9.47 (s, 1 H, indole-NH), 8.24 (d, $J = 1.8$ Hz, 1 H, H 7), 7.79 (dd, $J = 8.3, 2.1$ Hz, 1 H, H 5''), 7.71 (d, $J = 2.1$ Hz, 1 H, H 3''), 7.48 (d, $J = 8.3$ Hz, 1 H, H 6''), 7.42 (br s, 1 H, OCONH), 7.33 (s, 1 H, H 5), 7.21 (d, $J = 8.2$ Hz, 1 H, H 4), 6.93 (d, $J = 2.3$ Hz, 1 H, H 3'), 6.85 (s, 1 H, H 4'), 5.29 (s, 2 H, CH_2O), 4.62 (dd, $J = 10.8, 9.8$ Hz, 1 H, H 2), 4.46 (dd, $J = 10.8, 4.5$ Hz, 1 H, H 2), 4.25 (t, $J = 5.8$ Hz, 2 H, CH_2O), 4.07 (s, 3 H, OCH_3), 3.94 (s, 3 H, OCH_3), 3.88-3.93 (m, 5 H, OCH_3 , CH_2O), 3.79-3.85 (m, 2 H, H 3, CH_2Cl), 3.55 (dd, $J = 12.1, 10.3$ Hz, 1 H, CH_2Cl), 2.28 (br t, $J = 4.9$ Hz, 1 H, OH), 2.09-2.14 (m, 2 H, CH_2); ^{13}C NMR δ 160.5, 156.8, 153.1, 150.1, 148.5, 144.0, 140.5, 138.7, 138.6, 131.7, 130.8, 129.5, 129.3, 126.0, 125.8, 124.5, 123.5, 115.5, 114.6, 108.5, 106.7, 106.0, 66.0, 61.7, 61.5, 61.1, 59.4, 56.1, 54.7, 46.9, 43.2, 31.6; MS (FAB $^+$) m/z 671 (MH^+ , 1%), 669 (MH^+ , 3%), 391 (15), 149 (100); HRMS (FAB $^+$) calc. for $\text{C}_{32}\text{H}_{34}^{35}\text{ClN}_4\text{O}_{10}$ (MH^+) m/z 669.1964, found 669.1921; calc. for $\text{C}_{32}\text{H}_{34}^{37}\text{ClN}_4\text{O}_{10}$ (MH^+) m/z 669.1934, found 671.1875; Anal. ($\text{C}_{32}\text{H}_{33}\text{ClN}_4\text{O}_{10} \cdot \frac{1}{2}\text{H}_2\text{O}$) C, H, N.

30 Compound **80** was also prepared by treating a solution of **79** (96 mg, 0.13 mmol) in MeOH (5 mL) with 0.1 M HCl (2 mL) and stirring at 20 °C for 16 h. The solvent was evaporated and the residue partitioned between DCM (50 mL) and water (50 mL). The organic fraction

was washed with water (10 mL), brine (10 mL), dried, and the solvent evaporated. The residue was purified by chromatography, eluting with 40% EtOAc/DCM, to give **80** (69 mg, 79%) as a tan solid, spectroscopically identical with the sample prepared above.

5 Example 4E. Preparation of 2-(3-(hydroxypropoxy)-4-nitrobenzyl 1-(chloromethyl)-3-[(5,6,7-trimethoxy-1*H*-indol-2-yl)carbonyl]-2,3-dihydro-1*H*-benzo[*e*]indol-5-ylcarbamate (84).

Methyl 2-(3-({*tert*-butyl(dimethyl)silyl}oxy)propoxy)-4-nitrobenzoate (81). A mixture of methyl 2-hydroxy-4-nitrobenzoate (**66**) (1.82 g, 9.23 mmol) and K₂CO₃ (1.91 g, 13.85 mmol) in DMF (30 mL) was stirred at 20 °C for 30 min. A solution of 3-bromopropyl *tert*-butyl(dimethyl)silyl ether (3.50 g, 13.85 mmol) in DMF (10 mL) was added and the mixture stirred at 100 °C for 3 h. The mixture was poured into water (300 mL), extracted with EtOAc (3 × 100 mL) and the combined organic extract washed with water (2 × 100 mL), brine (50 mL), dried and the solvent evaporated. The residue was purified by chromatography, eluting with 10% EtOAc/light petroleum, to give **81** (3.19 g, 93%) as a pale yellow solid, mp (EtOAc) 36.5-37 °C; ¹H NMR δ 7.88 (d, *J* = 8.9 Hz, 1 H, H 6), 7.80-7.84 (m, 2 H, H 3, H 5), 4.24 (t, *J* = 6.0 Hz, 2 H, CH₂O), 3.92 (s, 3 H, OCH₃), 3.85 (t, *J* = 5.9 Hz, 2 H, CH₂O), 2.04-2.09 (m, 2 H, CH₂), 0.88 (s, 9 H, OSi(CH₃)₃), 0.04 (s, 6 H, OSi(CH₃)₂); ¹³C NMR δ 165.4 (CO₂), 158.7 (C 2), 150.7 (C 4), 132.0 (C 6), 126.1 (C 1), 114.8 (C 5), 107.7 (C 3), 64.0 (CH₂O), 59.0 (CH₂O), 52.5 (OCH₃), 32.0 (CH₂), 25.9 (SiC(CH₃)₃), 18.3 (SiC(CH₃)₃), -5.5 (Si(CH₃)₂); Anal. (C₁₇H₂₇NO₆Si) C, H, N.

[2-(3-({*tert*-Butyl(dimethyl)silyl}oxy)propoxy)-4-nitrophenyl]methanol (82). DIBALH (1 M in DCM, 16.5 mL, 16.5 mmol) was added to a stirred solution of ester **81** (1.85 g, 5.0 mmol) in THF (100 mL) at 5 °C and the solution stirred at 5 °C for 1 h. The solution was poured into a solution of potassium sodium tartrate (1 M, 100 mL) and the mixture stirred vigorously for 20 min. The mixture was extracted with EtOAc (3 × 100 mL), the combined organic fraction washed with water (100 mL), brine (50 mL), dried, and the solvent evaporated. The residue was purified by chromatography, eluting with 20% EtOAc/light petroleum, to give **82** (1.64 g, 94%) as a pale yellow solid, mp (EtOAc/light petroleum) 48-49 °C; ¹H NMR δ 7.84 (dd, *J* = 8.3, 2.1 Hz, 1 H, H 5), 7.71 (d, *J* = 2.1 Hz, 1 H, H 3), 7.51 (d, *J* = 8.3 Hz, 1 H, H 6), 4.76 (d, *J* = 6.3 Hz, 2 H, CH₂O), 4.21 (t, *J* = 6.1 Hz, 2 H, CH₂O),

- 68 -

3.82 (t, $J = 5.9$ Hz, 2 H, CH₂OSi), 2.40 (t, $J = 6.3$ Hz, 1 H, OH), 2.02-2.08 (m, 2 H, CH₂), 0.89 (s, 9 H, OSiC(CH₃)₃), 0.06 (s, 6 H, OSi(CH₃)₂); ¹³C NMR δ 156.5 (C 2), 148.2 (C 4), 136.7 (C 1), 127.8 (C 6), 115.9 (C 5), 105.8 (C 3), 65.5 (CH₂O), 60.8 (CH₂O), 59.3 (CH₂O), 32.0 (CH₂), 25.9 (OSiC(CH₃)₃), 18.3 (OSiC(CH₃)₃), -5.4 (OSi(CH₃)₂); Anal. (C₁₆H₂₇NO₅Si) C, H, N.

2-(3-[[*tert*-Butyl(dimethyl)silyl]oxy]propoxy)-4-nitrobenzyl 1-(chloromethyl)-3-[(5,6,7-trimethoxy-1*H*-indol-2-yl)carbonyl]-2,3-dihydro-1*H*-benzo[*e*]indol-5-ylcarbamate (83). A solution of triphosgene (15 mg, 51 μ mol) in DCM (2 mL) was added dropwise to a stirred solution of amine 1 [G. J. Atwell, W. R. Wilson, W. A. Denny, *Bioorg. Med. Chem. Lett.*, **1997**, 7, 1483] (60 mg, 129 μ mol) and Et₃N (40 μ L, 289 μ mol) in DCM (10 mL) and stirred at 20 °C for 2 h. A solution of alcohol 82 (54 mg, 159 μ mol) in DCM (2 mL) was added, followed by nBu₂Sn(OAc)₂ (2 drops) and the solution stirred at 20 °C for 24 h. The solvent was evaporated and the residue purified by chromatography, eluting with a gradient (0-10%) MeOH/EtOAc, to give 83 (72 mg, 67%) as a yellow solid mp (MeOH) 149-151 °C; ¹H NMR δ 9.42 (s, 1 H, indole-NH), 8.96 (s, 1 H, OCONH), 7.91 (d, $J = 8.4$ Hz, 1 H, H 6), 7.78-7.85 (m, 2 H, H 9, H 5"), 7.75 (d, $J = 1.7$ Hz, 1 H, H 3"), 7.53-7.59 (m, 2 H, H 8, H 6"), 7.47 (ddd, $J = 8.4, 7.4, 0.8$ Hz, 1 H, H 7), 7.08 (br s, 1 H, H 4), 7.02 (d, $J = 2.2$ Hz, 1 H, H 3'), 6.89 (s, 1 H, H 4'), 5.38 (s, 2 H, CH₂O), 4.82 (dd, $J = 10.7, 1.7$ Hz, 1 H, H 2), 4.69 (dd, $J = 10.7, 8.7$ Hz, 1 H, H 2), 4.21 (t, $J = 6.0$ Hz, 2 H, CH₂O), 4.17-4.20 (m, 1 H, CH₂Cl), 4.09 (s, 3 H, OCH₃), 3.99 (dd, $J = 11.3, 2.9$ Hz, 1 H, H 1), 3.95 (s, 3 H, OCH₃), 3.92 (s, 3 H, OCH₃), 3.83 (t, $J = 5.9$ Hz, 2 H, CH₂O), 3.49 (t, $J = 11.0$ Hz, 1 H, CH₂Cl), 2.02-2.08 (m, 2 H, CH₂), 0.88 (s, 9 H, OSiC(CH₃)₃), 0.04 (s, 6 H, OSi(CH₃)₂); MS (FAB⁺) m/z 833 (MH⁺, 25%), 835 (MH⁺, 12), 775 (5), 599 (5); HRMS (FAB⁺) calc. for C₄₂H₅₀³⁵ClN₄O₁₀Si (MH⁺) m/z 833.2985, found 833.3008; calc. for C₄₂H₅₀³⁷ClN₄O₁₀Si (MH⁺) m/z 835.2955, found 835.2982; Anal. (C₄₂H₄₉ClN₄O₁₀Si) C, H, N.

2-(3-Hydroxypropoxy)-4-nitrobenzyl 1-(chloromethyl)-3-[(5,6,7-trimethoxy-1*H*-indol-2-yl)carbonyl]-2,3-dihydro-1*H*-benzo[*e*]indol-5-ylcarbamate (84). 1 M HCl (0.2 mL, 200 μ mol) was added to a stirred solution of silyl ether 83 (64 mg, 77 μ mol) in MeOH (5 mL) and the solution stirred at 20 °C for 30 min. The solvent was evaporated and the residue dissolved in EtOAc (50 mL), washed with water (2 \times 50 mL), brine (25 mL), dried

and the solvent evaporated. The residue was purified by chromatography, eluting with a gradient (50-100%) of EtOAc/light petroleum, to give **84** (52 mg, 94%) as a tan solid, mp (EtOAc) 122-126 °C; ¹H NMR δ 9.51 (s, 1 H, indole-NH), 8.90 (s, 1 H, OCONH), 7.92 (d, *J* = 8.5 Hz, 1 H, H 6), 7.80 (d, *J* = 8.2 Hz, 1 H, H 5"), 7.77 (d, *J* = 8.3 Hz, 1 H, H 9), 7.73 (d, *J* = 1.8 Hz, 1 H, H 3"), 7.50-7.57 (m, 2 H, H 8, H 6"), 7.40-7.46 (m, 2 H, H 4, H 7), 6.99 (d, *J* = 2.2 Hz, 1 H, H 3'), 6.87 (s, 1 H, H 4'), 5.37 (d, *J* = 13.1 Hz, 1 H, CH₂O), 5.32 (d, *J* = 13.1 Hz, 1 H, CH₂O), 4.77 (dd, *J* = 10.8, 1.6 Hz, 1 H, H 2), 4.64 (dd, *J* = 10.8, 8.6 Hz, 1 H, H 2), 4.27 (t, *J* = 5.7 Hz, 2 H, CH₂O), 4.11-4.18 (m, 1 H, CH₂Cl), 4.09 (s, 3 H, OCH₃), 3.96 (s, 3 H, OCH₃), 3.91-3.95 (m, 3 H, H 1, CH₂O), 3.90 (s, 3 H, OCH₃), 3.44 (t, *J* = 10.9 Hz, 1 H, CH₂Cl), 2.75 (br s, 1 H, OH), 2.12-2.18 (m, 2 H, CH₂); ¹³C NMR δ 160.4 (CO), 157.2 (C 2"), 153.8 (OCONH), 150.2 (C 5'), 148.9 (C 4"), 141.6 (C 3a), 140.6 (C 6'), 138.9 (C 7'), 134.0 (C 5), 131.6 (C 1"), 130.1 (C 6"), 129.7 (C 2'), 129.6 (C 9a), 127.5 (C 8), 125.7 (C 7a'), 125.0 (C 7), 123.6 (C 3a'), 123.1 (C 9), 122.4 (C 6, C 9b), 121.6 (C 5a), 115.7 (C 5"), 112.2 (C 4), 106.6 (C 3'), 106.1 (C 3"), 97.7 (C 4'), 66.8 (CH₂O), 62.2 (CH₂O), 61.5 (OCH₃), 61.1 (OCH₃), 60.1 (CH₂O), 56.3 (OCH₃), 55.0 (C 2), 45.8 (CH₂Cl), 43.4 (C 1), 31.6 (CH₂); MS (FAB⁺) *m/z* 721 (MH⁺, 2%), 719 (MH⁺, 4); HRMS (FAB⁺) calc. for C₃₆H₃₅³⁵ClN₄O₁₀ (MH⁺) *m/z* 719.2120, found 719.2107; calc. for C₃₆H₃₅³⁷ClN₄O₁₀ (MH⁺) *m/z* 721.2091, found 721.2093; Anal. (C₃₆H₃₅ClN₄O₁₀) C, H, N.

20 Example 4F. Preparation of 2-(3-hydroxypropoxy)-4-nitrobenzyl doxorubicin carbamate (87).

4-Nitrophenyl 4-nitro-2-[3-(tetrahydro-2*H*-pyran-2-yloxy)propoxy]benzyl carbonate (85). A solution of 4-nitrophenylchloroformate (0.43 g, 2.1 mmol) in THF (10 mL) was added dropwise to a stirred solution of alcohol **75** (0.44 g, 1.4 mmol) and DIEA (0.49 mL, 2.8 mmol) in THF (40 mL) and the mixture stirred at 20 °C for 48 h. The solution was evaporated and the residue partitioned between EtOAc (100 mL) and water (100 mL). The organic fraction was washed with water (3 × 50 mL), brine (50 mL), dried, and the solvent evaporated. The residue was purified by chromatography, eluting with a gradient (10-50%) EtOAc/light petroleum, to give (i) starting material (176 mg, 40%); and (ii) **85** (0.38 g, 56%) as a pale yellow oil, ¹H NMR δ 8.30 (ddd, *J* = 9.2, 3.1, 2.1 Hz, 2 H, H 3, H 5), 7.87 (dd, *J* = 8.4, 2.1 Hz, 1 H, H 5'), 7.79 (d, *J* = 2.1 Hz, 1 H, H 3'), 7.57 (d, *J* = 8.4 Hz, 1 H, H 6'), 7.41 (ddd, *J* = 9.2, 3.1, 2.1 Hz, 2 H, H 2, H 6), 5.42 (s, 2 H, CH₂O), 4.58-4.61 (m, 1 H,

- 70 -

OCHO), 4.28 (t, $J = 6.3$ Hz, 2 H, CH₂O), 3.96 (dt, $J = 10.0, 6.0$ Hz, 1 H, CH₂O), 3.78-3.83 (m, 1 H, CH₂O), 3.59 (dt, $J = 10.0, 6.0$ Hz, 1 H, CH₂O), 3.45-3.52 (m, 1 H, CH₂O), 2.13-2.18 (m, 2 H, CH₂O), 1.79-1.86 (m, 1 H, CH₂), 1.67-1.76 (m, 1 H, CH₂), 1.48-1.60 (m, 4 H, 2 × CH₂); ¹³C NMR δ 157.0 (C 1), 155.4 (C 2'), 153.4 (OCONH), 149.2 (C 4'), 145.5 (C 4), 129.9 (C 1), 129.2 (C 6'), 125.3 (C 3, C 5), 121.7 (C 2, C 6), 115.6 (C 5'), 106.3 (C 6'), 99.1 (OCO), 66.1 (CH₂O), 65.3 (CH₂O), 63.5 (CH₂O), 60.4 (CH₂O), 30.6 (CH₂), 29.4 (CH₂), 25.4 (CH₂), 19.7 (CH₂); MS m/z 476 (M⁺, 2%), 459 (5), 392 (2), 210(30), 85 (100); HRMS calc. for C₂₂H₂₄N₂O₁₀ (M⁺) m/z 476.1431, found 476.1425.

- 10 **2-(3-Hydroxypropoxy)-4-nitrobenzyl 4-nitrophenyl carbonate (86).** A solution of ether 85 (207 mg, 0.47 mmol) in THF (20 mL) and 1 M HCl (5 mL) was stirred at 20 °C for 16 h. The solvent was evaporated and the residue partitioned between EtOAc (50 mL) and water (50 mL). The organic fraction was dried, the solvent evaporated, and the residue purified by chromatography, eluting with 50% EtOAc/light petroleum, to give 86 (125 mg, 68%) as a white solid, mp (EtOAc/light petroleum) 116-117 °C; ¹H NMR δ 8.29 (ddd, $J = 9.1, 3.2, 2.1$ Hz, 2 H, H 3, H 5), 7.88 (dd, $J = 8.3, 2.1$ Hz, 1 H, H 5'), 7.80 (d, $J = 2.1$ Hz, 1 H, H 3'), 7.58 (d, $J = 8.3$ Hz, 1 H, H 6'), 7.40 (ddd, $J = 9.1, 3.2, 2.1$ Hz, 2 H, H 2, H 6), 5.41 (s, 2 H, CH₂O), 4.30 (t, $J = 6.0$ Hz, 2 H, CH₂O), 3.90 (dt, $J = 5.4, 4.6$ Hz, 2 H, CH₂O), 2.10-2.15 (m, 2 H, CH₂), 1.65 (br s, 1 H, OH); ¹³C NMR δ 157.0 (C 2'), 155.3 (C 1), 152.3 (OCONH), 149.3 (C 4'), 145.5 (C 4), 129.8 (C 1'), 129.6 (C 6'), 125.4 (C 2, C 6), 121.7 (C 3, C 5), 115.8 (C 5'), 106.4 (C 3'), 66.2 (CH₂O), 65.3 (CH₂O), 59.5 (CH₂O), 31.7 (CH₂); Anal. (C₁₇H₁₆N₂O₉) C, H, N.

- 2-(3-Hydroxypropoxy)-4-nitrobenzyl doxorubicin carbamate (87).** A solution of carbonate 86 (41 mg, 104 μ mol) in DMF (2 mL) was added dropwise to a stirred solution of doxorubicin 13 (46 mg, 86 μ mol) and Et₃N (15 μ L, 104 μ mol) in DMF (5 mL) at 20 °C and the solution stirred for 16 h. The solvent was evaporated and the residue purified by chromatography, eluting with a gradient (0-10%) of MeOH/DCM, to give 87 (69 mg, 84%) as a red solid, mp (DCM) 154-160 °C; ¹H NMR [(CD₃)₂SO] δ 14.00 (s, 1 H, 6-OH), 13.24 (s, 1 H, 11-OH), 7.85-7.89 (m, 2 H, H 1, H 3), 7.80 (dd, $J = 8.3, 1.8$ Hz, 1 H, H 5''), 7.71 (d, $J = 1.8$ Hz, 1 H, H 3''), 7.62 (dd, $J = 6.6, 2.8$ Hz, 1 H, H 2), 7.50 (d, $J = 8.3$ Hz, 1 H, H 6''), 7.07 (d, $J = 8.0$ Hz, 1 H, OCONH), 5.42 (s, 1 H, 9-OH), 5.14 (br s, 1 H, H 1'), 5.05 (d, $J =$

- 71 -

18.4 Hz, 1 H, CH₂O), 4.99 (d, J = 18.4 Hz, 1 H, CH₂O), 4.92 (br s, 1 H, H 7), 4.85 (t, J = 6.0 Hz, 1 H, 14-OH), 4.74 (d, J = 5.8 Hz, 1 H, 4'-OH), 4.58 (d, J = 6.0 Hz, 2 H, H 14), 4.55 (t, J = 5.3 Hz, 1 H, H 5'), 4.14-4.20 (m, 2 H, CH₂O), 3.97 (s, 3 H, 4-OCH₃), 3.69-3.76 (m, 1 H, H 3'), 3.54 (dt, J = 6.0, 5.7 Hz, 2 H, CH₂O), 3.48 (br s, 1 H, H 4'), 3.30 (br s, 1 H, OH),
 5 2.98 (d, J = 18.2 Hz, 1 H, H 10), 2.90 (d, J = 18.2 Hz, 1 H, H 10), 2.22 (br d, J = 14.4 Hz, 1 H, H 8), 2.09 (dd, J = 14.4, 5.5 Hz, 1 H, H 8), 1.88-1.92 (m, 1 H, H 2'), 1.82-1.87 (m, 2 H, CH₂), 1.50 (dd, J = 12.4, 3.7 Hz, 1 H, H 2'), 1.13 (d, J = 6.4 Hz, 3 H, H 6'); ¹³C NMR [(CD₃)₂SO] δ 213.7 (C 13), 186.4 (C 5), 186.3 (C 12), 160.7 (C 4), 156.0 (C 2'), 155.9 (C 6), 154.9 (C 11), 154.4 (OCONH), 147.8 (C 4'), 136.1 (C 2), 135.4 (C 12a), 134.5 (C 6a),
 10 134.0 (C 10a), 133.2 (C 1'), 127.8 (C 6'), 119.9 (C 4a), 119.6 (C 1), 118.9 (C 3), 115.3 (C 5'), 110.6 (C 5a), 110.5 (C 11a), 105.8 (C 3'), 100.2 (C 1'), 74.8 (C 9), 69.8 (C 7), 67.9 (C 4'), 66.6 (C 5'), 65.6 (CH₂O), 63.6 (C 14), 59.8 (CH₂O), 57.0 (CH₂O), 56.5 (4-OCH₃), 47.2 (C 3'), 36.5 (C 8), 32.0 (C 10), 31.7 (CH₂), 29.7 (C 2'), 16.9 (C 6'); MS (FAB⁺) m/z 797 (MH⁺, 0.3%); HRMS (FAB⁺) calc. for C₃₈H₄₀N₂O₁₇ (MH⁺) m/z 797.2405, found 797.2953;
 15 Anal. (C₃₈H₄₀N₂O₁₇·½H₂O) C, H, N.

Example 4G. Preparation of 2-(3-hydroxypropoxy)-4-nitrobenzyl bis(3-[(5-methyl-4-acridinyl)carbonyl]amino}propyl)carbamate (91).

4-Nitro-2-[3-(tetrahydro-2H-pyran-2-yloxy)propoxy]benzyl bis{3-

20 [(trifluoroacetyl)amino]propyl}carbamate (88). A solution of alcohol 75 (623 mg, 2.0 mmol) and DIEA (0.40 mL, 2.4 mmol) in DCM (8 mL) was added dropwise to a solution of triphosgene (208 mg, 0.70 mmol) in DCM (6 mL) over 30 minutes at 5 °C and stirred for 1 h. The reaction mixture was added dropwise to a suspension of bistrifluoroacetamide
 25 47 (880 mg, 2.0 mmol) and DIEA (0.76 mL, 4.8 mmol) in DCM (8 mL) and the solution stirred at 20 °C for 16 h. The solvent was evaporated and the residue purified by chromatography, eluting with 50% EtOAc/petroleum ether, to give 88 (804 mg, 61%) as a colorless oil, ¹H NMR [(CD₃)₂SO] δ 7.85 (dd, J = 8.0, 2.0 Hz, 1 H, H 5'), 7.80 (br s, 1 H, CONH), 7.76 (d, J = 2.0 Hz, 1 H, H 3'), 7.44 (d, J = 8.0 Hz, 1 H, H 6'), 6.81 (br s, 1 H, CONH), 5.25 (s, 2 H, CH₂O), 4.58-4.61 (m, 1 H), 4.22-4.26 (m, 2 H), 3.91-3.98 (m, 1 H),
 30 3.79-3.86 (m, 1 H), 3.56-3.63 (m, 1 H), 3.46-3.53 (m, 1 H), 3.28-3.40 (m, 8 H), 2.04-2.16 (m, 2 H), 1.72-1.87 (m, 6 H), 1.50-1.63 (m, 4 H); ¹³C NMR [(CD₃)₂SO] δ 157.0, 156.9, 148.9, 131.5, 129.4, 115.7, 105.3, 99.2, 65.9, 63.7, 62.6, 62.5, 44.3 (2), 37.4, 36.1, 30.7,

29.4, 28.1, 27.1, 25.3, 19.6, $2 \times \text{CF}_3\text{CO}$ not observed; HRMS (FAB⁺) calc. for $\text{C}_{26}\text{H}_{34}\text{F}_6\text{N}_4\text{O}_9$ (M^+) m/z 660.2230; found 660.2234

4-Nitro-2-[3-(tetrahydro-2H-pyran-3-yloxy)propoxy]benzyl bis(3-[(5-methyl-4-acridinyl)carbonyl]amino)propyl)carbamate (90). A solution of carbamate **88** (165 mg, 0.25 mmol), Cs_2CO_3 (1.0 g, 3.0 mmol) and water (1 mL) in methanol (4 mL) was stirred at 20 °C for 8 h. The pH was adjusted to 10, water (50 mL) added, the solution was extracted with DCM (3×50 mL). The combined organic fraction was dried, and the solvent was evaporated to give crude 4-nitro-2-[3-(tetrahydro-2H-pyran-2-yloxy)propoxy]benzyl bis(3-aminopropyl)carbamate (**89**). 4-(1H-Imidazol-1-ylcarbonyl)-5-methylacridine (**50**) [S. A. Gamage, J. A. Spicer, G. J. Atwell, G. J. Finlay, B. C. Baguley, W. A. Denny, *J. Med. Chem.*, **1999**, *42*, 2383-2393] (144 mg, 0.50 mmol) was added to a solution of carbamate (**89**) in THF (10 mL) at 5 °C and the reaction mixture was stirred at 20 °C for 8 h. The solvent was evaporated, and the residue was purified by chromatography on alumina-90, eluting with 1%MeOH/55%EtOAc/DCM, to give **90** (183 mg, 88%) as a yellow solid, mp (EtOAc/DCM) 80-81 °C; ^1H NMR δ 11.90 (s, 1 H, NH), 11.83 (s, 1 H, NH), 8.88-8.92 (m, 2 H), 8.62-8.72 (m, 2 H), 8.02-8.05 (m, 2 H), 7.74-7.83 (m, 2 H), 7.50-7.63 (m, 4 H), 7.36-7.45 (m, 2 H), 7.30 (d, $J = 2.0$ Hz, 1 H, H 3''), 7.02 (d, $J = 8.4$ Hz, 1 H, H 6''), 6.90 (dd, $J = 8.4, 2.0$ Hz, 1 H, H 5''), 5.01 (s, 2 H, CH_2O), 4.56 (s, 1 H), 4.00-3.50 (m, 14 H), 2.83 (s, 3 H, CH_3), 2.71 (s, 3 H, CH_3), 2.14-1.50 (m, 12 H); ^{13}C NMR δ 166.1, 155.6, 155.5, 147.5 (2), 147.0 (2), 145.2 (2), 137.9 (2), 135.8, 135.4, 135.2 (2), 132.6 (2), 132.3 (2), 131.0 (2), 128.3, 128.0, 126.5, 126.4, 126.2, 126.1 (2), 125.7 (2), 125.3 (2), 114.9, 104.9, 99.1, 65.6, 63.6, 62.5, 61.6, 45.9, 45.1, 37.7, 37.1, 30.7, 29.3, 29.4, 28.6, 25.4, 19.7, 18.9, 18.8, 14.2; Anal. ($\text{C}_{52}\text{H}_{54}\text{N}_6\text{O}_9 \cdot \frac{1}{2}\text{H}_2\text{O}$) C, H, N.

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2-(3-Hydroxypropoxy)-4-nitrobenzyl bis(3-[(5-methyl-4-acridinyl)carbonyl]amino)propyl)carbamate dihydrochloride (91). A solution of ether **90** (51 mg, 56 μmol) and HCl (1 M, 1.5 mL) in MeOH (10 mL) was stirred at 20 °C for 4 hrs. The solvent was evaporated and the residue was recrystallized to give **91** (46 mg, 92%) as a yellow solid, mp (MeOH/EtOAc/light petroleum) 143-145°C; ^1H NMR [$(\text{CD}_3)_2\text{SO}$] δ 11.23 (s, 2 H, $2 \times \text{NH}$), 9.17 (s, 1 H), 9.11 (s, 1 H), 8.70 (br s, 2 H), 8.24 (br s, 2 H), 7.96 (br s, 2 H), 7.66 (br s, 4 H), 7.51 (br s, 2 H), 7.32 (d, $J = 2.0$ Hz, 1 H, H 3''), 7.02 (d, $J = 8.4$

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- 73 -

Hz, 1 H, H 6''), 6.97 (dd, $J = 8.4, 2.0$ Hz, 1 H, H 5''), 4.80 (s, 2 H, CH₂O), 3.92-3.96 (m, 2 H), 3.50-3.53 (m, 10 H), 2.72 (s, 3 H, CH₃), 2.63 (s, 3 H, CH₃), 2.01-2.04 (m, 4 H, 2 × CH₂), 1.75-1.82 (m, 2 H); HRMS (FAB⁺) calc. for (C₄₇H₄₆N₆O₈) (M⁺) m/z 823.3455, found 823.3467; Anal. (C₄₇H₄₆N₆O₈·2HCl·2½H₂O) C, H, N.

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Example 4H. Preparation of 4-nitro-2-[3-(phosphonooxy)propoxy]benzyl 1-(chloromethyl)-3-[(5,6,7-trimethoxy-1H-indol-2-yl)carbonyl]-2,3-dihydro-1H-benzo[e]indol-5-ylcarbamate (93).

- 2-(3-[[Di(*tert*-butoxy)phosphoryl]oxy]propoxy)-4-nitrobenzyl 1-(chloromethyl)-3-
 10 [(5,6,7-trimethoxy-1H-indol-2-yl)carbonyl]-2,3-dihydro-1H-benzo[e]indol-5-ylcarbamate (92). Tetrazole (40 mg, 567 μmol) was added to a stirred solution of alcohol 84 (Example 4E) 136 mg, 189 μmol) and di-*tert*-butyl diethylphosphoramidite (68 μL, 227 mmol) in THF (10 mL) under N₂ and the solution stirred at 20 °C for 4 h. The solution was cooled to -40 °C and a dried (Na₂SO₄) solution of MCPBA (70 %, 65 mg, 265 μmol) in
 15 DCM (3 mL) added. The solution was stirred at -40 °C for 10 min and a solution of 10% NaHSO₄ (10 mL) added and the mixture stirred for 10 min. The mixture was extracted with diethyl ether (80 mL), the organic fraction washed with 10% aq. NaHSO₄ (10 mL), sat. aq. KHCO₃ (10 mL), dried, and the solvent evaporated. The residue was purified by chromatography, eluting with a gradient (10-50%) EtOAc/light petroleum, to give 92 (160
 20 mg, 93%) as an oil, ¹H NMR δ 9.42 (s, 1 H, indole-NH), 8.89 (s, 1 H, OCONH), 8.15 (d, $J = 8.5$ Hz, 1 H, H 6), 7.83 (dd, $J = 8.2, 2.1$ Hz, 1 H, H 5''), 7.79 (s, 1 H, H 4), 7.75 (d, $J = 8.2$ Hz, 1 H, H 6''), 7.73 (d, $J = 2.1$ Hz, 1 H, H 3''), 7.60 (d, $J = 8.3$ Hz, 1 H, H 9), 7.53 (ddd, $J = 8.3, 7.1, 0.8$ Hz, 1 H, H 8), 7.39 (ddd, $J = 8.5, 7.1, 0.8$ Hz, 1 H, H 7), 7.01 (d, $J = 2.1$ Hz, 1 H, H 3'), 6.89 (s, 1 H, H 4'), 5.34 (s, 2 H, CH₂O), 4.81 (dd, $J = 10.7, 1.8$ Hz, 1 H, H 2),
 25 4.64 (dd, $J = 10.7, 8.6$ Hz, 1 H, H 2), 4.35 (dt, $J = 5.6, 5.5$ Hz, 2 H, CH₂O), 4.29 (t, $J = 5.6$ Hz, 2 H, CH₂O), 4.15-4.20 (m, 1 H, H 1), 4.10 (s, 3 H, OCH₃), 3.98 (dd, $J = 11.2, 2.9$ Hz, 1 H, CH₂Cl), 3.95 (s, 3 H, OCH₃), 3.92 (s, 3 H, OCH₃), 3.45 (dd, $J = 10.9, 10.8$ Hz, 1 H, CH₂Cl), 2.19-2.25 (m, 2 H, CH₂), 1.35 (2s, 18 H, 2 × OC(CH₃)₃); MS (FAB⁺) m/z 913 (MH⁺, 0.4%), 911 (MH⁺, 0.8); HRMS (FAB⁺) calc. for C₄₄H₅₃³⁵ClN₄O₁₃P (MH⁺) m/z
 30 911.3035, found 911.3003; calc. for C₄₄H₅₃³⁷ClN₄O₁₃P (MH⁺) m/z 913.3006, found 913.3002.

4-Nitro-2-[3-(phosphonooxy)propoxy]benzyl 1-(chloromethyl)-3-[(5,6,7-trimethoxy-1*H*-indol-2-yl)carbonyl]-2,3-dihydro-1*H*-benzo[*e*]indol-5-ylcarbamate (93).

Trifluoroacetic acid (130 μ L, 1.64 μ mol) was added to a stirred solution of ester 92 (150 mg, 165 μ mol) in DCM (5 mL) and the solution stirred at 20 °C for 1 h. The solvent was
 5 evaporated, and the residue azeotroped with benzene (3 \times 1 mL) to give 93 (88 mg, 66%) as a gum, ¹H NMR δ 11.47 (s, 1 H, indole-NH), 9.94 (s, 1 H, OCONH), 8.57 (br s, 3 H, H 4, 2 \times OH), 8.11 (d, *J* = 8.5 Hz, 1 H, H 6), 7.98 (d, *J* = 8.3 Hz, 1 H, H 9), 7.91 (d, *J* = 8.3 Hz, 1 H, H 5"), 7.81 (d, *J* = 1.8 Hz, 1 H, H 3"), 7.69 (d, *J* = 8.3 Hz, 1 H, H 6"), 7.58 (ddd, *J* = 8.3, 7.2, 0.7 Hz, 1 H, H 8), 7.47 (ddd, *J* = 8.5, 7.2, 0.7 Hz, 1 H, H 7), 7.10 (d, *J* = 2.2 Hz,
 10 1 H, H 3'), 6.97 (s, 1 H, H 4'), 5.29 (s, 2 H, CH₂O), 4.80 (dd, *J* = 10.8, 9.4 Hz, 1 H, H 2), 4.53 (dd, *J* = 10.8, 1.7 Hz, 1 H, H 2), 4.31–4.37 (m, 1 H, H 1), 4.27 (t, *J* = 6.1 Hz, 2 H, CH₂O), 4.07 (dd, *J* = 11.2 Hz, 1 H, CH₂Cl), 4.03 (dt, *J* = 7.1, 6.2 Hz, 2 H, CH₂O), 3.91–3.95 (m, 4 H, OCH₃, CH₂Cl), 3.82 (s, 3 H, OCH₃), 3.80 (s, 3 H, OCH₃), 2.02–2.10 (m, 2 H, CH₂);
¹³C NMR δ 160.2 (CO), 156.2 (C 2"), 154.4 (OCONH), 149.1 (C 5'), 148.1 (C 4"), 141.4 (C
 15 3a), 139.9 (C 6'), 139.0 (C 7'), 134.4 (C 5), 132.8 (C 1"), 130.8 (C 9a), 129.4 (C 2'), 128.5 (C 6"), 127.1 (C 8), 125.4 (C 5a, C 7a'), 124.3 (C 7), 123.8 (C 9), 123.2 (C 6), 123.1 (C 3a'), 122.0 (C 9b), 115.4 (C 5"), 113.0 (C 4), 106.2 (C 3'), 106.0 (C 3"), 98.0 (C 4'), 65.1 (CH₂O), 61.3 (CH₂O), 61.0 (OCH₃), 60.9 (OCH₃), 60.7 (CH₂O), 55.9 (OCH₃), 54.9 (C 2), 47.5 (CH₂Cl), 41.2 (C 1), 29.6 (CH₂); MS (FAB⁺) *m/z* 801 (MH⁺, 0.5%), 799 (MH⁺, 0.8);
 20 HRMS (FAB⁺) calc. for C₃₆H₃₇³⁵ClN₄O₁₃P (MH⁺) *m/z* 799.1783, found 799.1757; calc. for C₃₆H₃₇³⁷ClN₄O₁₃P (MH⁺) *m/z* 801.1754, found 801.1730.

Example 4I. Preparation of 2-(2,3-dihydroxypropoxy)-4-nitrobenzyl 1-(chloromethyl)-3-[(5,6,7-trimethoxy-1*H*-indol-2-yl)carbonyl]-2,3-dihydro-1*H*-benzo[*e*]indol-5-ylcarbamate (99).

Methyl 4-nitro-2-(2-oxiranylmethoxy)benzoate (94). A mixture of methyl 4-nitrosalicylate (66) (0.99 g, 5.02 mmol) and K₂CO₃ (1.04 g, 7.53 mmol) in DMF (25 mL) was stirred at 20 °C for 20 min. Epichlorohydrin (0.59 mL, 7.53 mmol) was added and the
 30 mixture stirred at 100 °C for 2 h. The mixture was poured into water, extracted with EtOAc (3 \times 100 mL), the combined organic extracts washed with water (2 \times 50 mL), brine (50 mL), dried, and the solvent evaporated. The residue was purified by chromatography, eluting with a gradient (20–50%) EtOAc/light petroleum, to give (i) starting material (0.18

g, 18%) and (ii) **94** (0.75 g, 59 %) as a colourless solid, mp (EtOAc/light petroleum) 62-63 °C; ¹H NMR δ 7.91 (dd, *J* = 7.7, 1.0 Hz, 1 H, H 5), 7.84-7.86 (m, 2 H, H 3, H 6), 4.49 (dd, *J* = 11.2, 2.4 Hz, 1 H, H 3'), 4.14 (dd, *J* = 11.2, 5.2 Hz, 1 H, H 3'), 3.94 (s, 3 H, OCH₃), 3.40-3.44 (m, 1 H, H 2'), 2.91-2.97 (m, 2 H, H 1'); ¹³C NMR δ 165.0 (CO₂), 158.1 (C 2), 150.6 (C 4), 132.3 (C 6), 126.1 (C 1), 115.6 (C 5), 108.4 (C 3), 69.6 (OCH₃), 52.6 (CH₂O), 49.7 (CH₂O), 44.3 (C 2'); MS (CI, NH₃) *m/z* 295 (M+CH₃CN⁺, 70%), 259 (MH⁺, 100%); Anal. (C₁₁H₁₁NO₆) C, H, N.

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Methyl 2-(2,3-dihydroxypropoxy)-4-nitrobenzoate (95). Perchloric acid (1 mL) and water (3 mL) was added to a stirred solution of **94** (205 mg, 0.81 mmol) in THF (20 mL) and the solution stirred at 20 °C for 16 h. The solvent was evaporated and the residue partitioned between EtOAc (50 mL) and water (50 mL). The organic fraction was washed with water (50 mL), brine (25 mL), dried, and the solvent evaporated. The residue was purified by chromatography, eluting with 70% EtOAc/light petroleum, to give **95** (172 mg, 78%) as an oil which solidified on standing, mp 60-65 °C; ¹H NMR δ 8.02 (d, *J* = 8.5 Hz, 1 H, H 6), 7.87 (dd, *J* = 8.5, 2.0 Hz, 1 H, H 5), 7.84 (d, *J* = 2.0 Hz, 1 H, H 3), 4.38 (dd, *J* = 9.3, 5.4 Hz, 1 H, H 3'), 4.23 (dd, *J* = 9.3, 5.4 Hz, 1 H, H 3'), 4.10-4.14 (m, 1 H, H 2'), 3.95 (s, 3 H, OCH₃), 3.88 (br d, *J* = 4.1 Hz, 2 H, H 1'), 3.05 (br s, 1 H, OH), 1.95 (br s, 1 H, OH); ¹³C NMR δ 164.8 (CO₂), 159.1 (C 2), 151.0 (C 4), 132.8 (C 6), 124.9 (C 1), 115.6 (C 5), 108.8 (C 3), 73.0 (CH₂O), 69.2 (C 2'), 63.2 (CH₂O), 52.8 (OCH₃); MS (CI, NH₃) *m/z* 272 (MH⁺, 1%), 240 (50%), 165 (100); HRMS (CI, NH₃) calc. for C₁₁H₁₄NO₇ (MH⁺) *m/z* 272.0770, found 272.0766. Anal. (C₁₁H₁₃NO₇) C, H, N.

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Methyl 2-[(2,2-dimethyl-1,3-dioxolan-4-yl)methoxy]-4-nitrobenzoate (96). 2,2-Dimethoxypropane (0.91 mL, 7.37 mmol) was added dropwise to a stirred solution of diol **95** (400 mg, 1.47 mmol) and PPTS (37 mg, 0.15 mmol) in DMF (20 mL) under N₂ and stirred at 20 °C for 24 h. The solvent was evaporated and the residue partitioned between EtOAc (100 mL) and water (100 mL). The organic fraction was washed with water (50 mL), brine (25 mL), dried, and the solvent evaporated. The residue was purified by chromatography, eluting with 30% EtOAc/light petroleum, to give **96** (458 mg, 100%) as a yellow oil, ¹H NMR δ 7.90 (d, *J* = 8.2 Hz, 1 H, H 6), 7.84-7.88 (m, 2 H, H 3, H 5), 4.49-4.54 (m, 1 H, H 4''), 4.25 (dd, *J* = 9.6, 4.6 Hz, 1 H, H 5''), 4.19 (dd, *J* = 8.5, 6.4 Hz, 1 H, H 2'), 4.15 (dd, *J* = 9.6, 4.6 Hz, 1 H, H 5''), 4.03 (dd, *J* = 8.5, 5.8 Hz, 1 H, H 2'), 3.94 (s, 3 H, OCH₃), 1.46 (s, 3 H, CH₃), 1.41 (s, 3 H, CH₃); ¹³C NMR δ 165.1 (CO₂), 158.2 (C 2), 150.6

35

- 76 -

(C 4), 132.2 (C 6), 126.4 (C 1), 115.5 (C 5), 109.9 (C 2''), 108.4 (C 3), 73.6 (C 4''), 69.8 (CH₂O), 66.5 (CH₂O), 52.6 (OCH₃), 26.6 (CH₃), 25.3 (CH₃); MS (CI, NH₃) *m/z* 312 (MH⁺, 15%), 296 (95), 101 (95), 71 (100); HRMS (CI, NH₃) calc. for C₁₄H₁₈NO₇ (MH⁺) *m/z* 312.1083, found 312.1092.

5

{2-[(2,2-Dimethyl-1,3-dioxolan-4-yl)methoxy]-4-nitrophenyl}methanol (97). DIBALH (1 M in DCM, 5.1 mL, 5.1 mmol) was added to a stirred solution of ester 96 (457 mg, 1.47 mmol) in THF (50 mL) at 5 °C and the solution stirred at 5 °C for 1 h. The solution was poured into a solution of potassium sodium tartrate (1 M, 100 mL) and the mixture stirred vigorously for 20 min. The mixture was extracted with EtOAc (3 × 50 mL), the combined organic fraction washed with water (50 mL), brine (50 mL), dried, and the solvent evaporated. The residue was purified by chromatography, eluting with 20% EtOAc/light petroleum, to give 97 (385 mg, 92%) as a white solid, mp (EtOAc/light petroleum) 90-92 °C; ¹H NMR δ 7.87 (dd, *J* = 8.2, 2.1 Hz, 1 H, H 5), 7.72 (d, *J* = 2.1 Hz, 1 H, H 3), 7.50 (d, *J* = 8.2 Hz, 1 H, H 6), 4.82 (d, *J* = 14.1 Hz, 1 H, CH₂O), 4.70 (d, *J* = 14.1 Hz, 1 H, CH₂O), 4.51-4.57 (m, 1 H, H 4''), 4.23 (dd, *J* = 9.8, 4.0 Hz, 1 H, H 5''), 4.19 (dd, *J* = 9.8, 5.4 Hz, 1 H, H 2'), 4.11 (dd, *J* = 9.8, 5.4 Hz, 1 H, H 5''), 3.95 (dd, *J* = 8.7, 5.4 Hz, 1 H, H 2'), 3.25 (br s, 1 H, OH), 1.48 (s, 3 H, CH₃), 1.41 (s, 3 H, CH₃); ¹³C NMR δ 156.5 (C 2), 148.2 (C 4), 137.2 (C 1), 128.6 (C 6), 116.6 (C 5), 110.0 (C 2'') 106.4 (C 3), 73.7 (CH₂O), 69.7 (CH₂O), 66.0 (CH₂O), 61.0 (CH₂O), 26.6 (CH₃), 25.0 (CH₃); MS *m/z* 283 (M⁺, 3%), 268 (20), 225 (30), 101 (100); HRMS calc for C₁₃H₁₇NO₆ (M⁺) *m/z* 283.1056, found 283.1055; Anal. (C₁₃H₁₇NO₆) C, H, N.

25 2-[(2,2-Dimethyl-1,3-dioxolan-4-yl)methoxy]-4-nitrobenzyl 1-(chloromethyl)-3-[(5,6,7-trimethoxy-1*H*-indol-2-yl)carbonyl]-2,3-dihydro-1*H*-benzo[*e*]indol-5-ylcarbamate (98). A solution of triphosgene (22 mg, 75 μmol) in DCM (3 mL) was added dropwise to a stirred solution of amin 1 [G. J. Atwell, W. R. Wilson, W. A. Denny, *Bioorg. Med. Chem. Lett.*, 1997, 7, 1483] (100 mg, 215 μmol) and Et₃N (60 μL, 429 μmol) in DCM (10 mL) and stirred at °C for 2 h. A solution of alcohol 97 (73 mg, 256 μmol) in DCM (3 mL) was added, followed by nBu₂Sn(OAc)₂ (2 drops) and the solution stirred at 20 °C for 24 h. The solvent was evaporated and the residue purified by chromatography, eluting with 20%EtOAc/DCM, to give 98 (160 mg, 96%) as a gum; ¹H NMR δ 9.44 (s, 1 H, indole-NH), 8.94 (s, 1 H, OCONH), 7.92 (d, *J* = 8.5 Hz, 1 H, H 6), 7.87 (dd, *J* = 8.2, 2.1 Hz, 1 H,

- 77 -

H 5"), 7.81 (d, $J = 8.2$ Hz, 1 H, H 9), 7.73 (d, $J = 2.1$ Hz, 1 H, H 3"), 7.58 (ddd, $J = 8.2, 7.3, 0.7$ Hz, 1 H, H 8), 7.45-7.51 (m, 2 H, H 7, H 6"), 7.13 (br s, 1 H, H 4), 7.02 (d, $J = 2.2$ Hz, 1 H, H 3'), 6.89 (s, 1 H, H 4'), 5.38 (s, 2 H, CH₂O), 4.83 (dd, $J = 10.8, 1.7$ Hz, 1 H, H 2), 4.69 (dd, $J = 10.8, 8.7$ Hz, 1 H, H 2), 4.50-4.56 (m, 1 H, H 4'''), 4.23 (dd, $J = 9.8, 4.0$ Hz, 1 H, H 5'''), 4.15-4.20 (m, 2 H, H 1, H 2'''), 4.09-4.14 (m, 4 H, OCH₃, H 5'''), 3.95-4.00 (m, 5 H, OCH₃, CH₂Cl, H 2'''), 3.92 (s, 3 H, OCH₃), 3.50 (dd, $J = 10.9, 10.8$ Hz, 1 H, CH₂Cl), 1.45 (s, 3 H, CH₃), 1.39 (s, 3 H, CH₃); MS (FAB⁺) m/z 777 (MH⁺, 10%), 775 (MH⁺, 35); HRMS (FAB⁺) calc. for C₃₉H₄₀³⁵ClN₄O₁₁ (MH⁺) m/z 775.2381, found 777.2379; calc. for C₃₉H₄₀³⁷ClN₄O₁₁ (MH⁺) m/z 777.2535, found 777.2354.

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2-(2,3-Dihydroxypropoxy)-4-nitrobenzyl 1-(chloromethyl)-3-[(5,6,7-trimethoxy-1H-indol-2-yl)carbonyl]-2,3-dihydro-1H-benzo[e]indol-5-ylcarbamate (99). 1 M HCl (1 mL) was added to a stirred suspension of 98 (160 mg, 206 μ mol) in THF (20 mL) and the mixture stirred at 20 °C for 16 h. The mixture was evaporated and the residue partitioned between DCM (50 mL) and water (50 mL). The organic fraction was washed with water (30 mL), brine (30 mL), dried, and the solvent evaporated. The residue was purified by chromatography, eluting with 50%EtOAc/DCM, to give 99 (87 mg, 56%) as a white solid, mp (MeOH/iPr₂O) 147-149 °C; ¹H NMR [(CD₃)₂SO] δ 11.46 (s, 1 H, indole-NH), 9.90 (s, 1 H, OCONH), 8.56 (s, 1 H, H 4), 8.12 (d, $J = 8.5$ Hz, 1 H, H 6), 7.98 (d, $J = 8.3$ Hz, 1 H, H 9), 7.92 (dd, $J = 8.3, 1.9$ Hz, 1 H, H 5"), 7.83 (d, $J = 1.9$ Hz, 1 H, H 3"), 7.69 (d, $J = 8.3$ Hz, 1 H, H 6"), 7.59 (dd, $J = 8.2, 7.6$ Hz, 1 H, H 8), 7.49 (dd, $J = 8.5, 7.6$ Hz, 1 H, H 7), 7.09 (d, $J = 2.1$ Hz, 1 H, H 3'), 6.98 (s, 1 H, H 4'), 5.33 (s, 2 H, CH₂O), 5.07 (d, $J = 5.2$ Hz, 1 H, OH), 4.81 (dd, $J = 11.0, 9.7$ Hz, 1 H, H 2), 4.73 (t, $J = 5.7$ Hz, 1 H, H 3'''), 4.53 (dd, $J = 11.0, 3.5$ Hz, 1 H, H 2), 4.32-4.37 (m, 1 H, H 1), 4.24 (dd, $J = 10.0, 3.9$ Hz, 1 H, CH₂Cl), 4.09-4.13 (m, 1 H, H 2'''), 4.02-4.06 (m, 1 H, H 3'), 3.93-3.96 (m, 4 H, OCH₃, CH₂Cl), 3.84-3.89 (m, 1 H, OH), 3.83 (s, 3 H, OCH₃), 3.81 (s, 3 H, OCH₃), 3.51 (t, $J = 5.7$ Hz, 2 H, H 1'''); ¹³C NMR [(CD₃)₂SO] δ 160.2 (CO), 156.2 (C 2"), 154.3 (OCONH), 149.2 (C 5'), 148.0 (C 4"), 141.5 (C 3a), 139.9 (C 6'), 139.0 (C 7'), 134.3 (C 5), 133.0 (C 1"), 130.7 (C 9a), 129.4 (C 2'), 128.0 (C 6"), 127.1 (C 8), 125.4 (C 5a, C 7a), 124.3 (C 7), 123.9 (C 9), 123.3 (C 6), 123.1 (C 3a'), 122.0 (C 9b), 115.4 (C 5"), 113.0 (C 4), 106.3 (C 3'), 106.2 (C 3"), 98.0 (C 4'), 70.7 (CH₂O), 69.7 (CHOH), 62.4 (CH₂O), 61.0 (OCH₃), 60.9 (OCH₃), 60.7 (CH₂O), 55.9 (OCH₃), 54.9 (C 2), 47.5 (CH₂Cl), 41.4 (C 1); MS (FAB⁺) m/z 737 (MH⁺, 3%), 735 (MH⁺, 8); HRMS (FAB⁺) calc. for C₃₆H₃₆³⁵ClN₄O₁₁ (MH⁺) m/z 735.2069, found

- 78 -

735.2050; calc. for $C_{36}H_{36}^{37}ClN_4O_{11}$ (MH^+) m/z 737.2040, found 737.2000; Anal. ($C_{36}H_{35}ClN_4O_{11} \cdot CH_3OH$) C, H, N.

Example 4J. Preparation of 2-[3-(dimethylamino)propoxy]-4-nitrobenzyl 1-

5 (chloromethyl)-3-[(5,6,7-trimethoxy-1*H*-indol-2-yl)carbonyl]-2,3-dihydro-1*H*-benzo[*e*]indol-5-ylcarbamate (**102**).

Methyl 2-[3-(dimethylamino)propoxy]-4-nitrobenzoate (**100**). A mixture of methyl 2-hydroxy-4-nitrobenzoate (**66**) (1.03 g, 5.22 mmol) and K_2CO_3 (2.17 g, 15.67 mmol) in DMF (30 mL) was stirred at 20 °C for 30 min. A solution of *N*-(3-chloropropyl)-*N,N*-dimethylamine (1.24 g, 7.83 mmol) in DMF (10 mL) was added and the mixture stirred at 100 °C for 3 h. The mixture was poured into water (300 mL), extracted with EtOAc (3 × 100 mL) and the combined organic extract washed with water (2 × 100 mL), brine (50 mL), dried and the solvent evaporated. The residue was purified by chromatography, eluting with a gradient (20-50%) of EtOAc/light petroleum, to give **100** (1.05 g, 71%) as a pale yellow oil which was stored as the HCl salt, mp (EtOAc) 175-177 °C; 1H NMR [$(CD_3)_2SO$] δ 10.90 (br s, 1 H, NHCl), 7.87-7.93 (m, 3 H, H 3, H 5, H 6), 4.32 (t, J = 6.0 Hz, 2 H, CH_2O), 3.89 (s, 3 H, OCH_3), 3.18-3.23 (m, 2 H, CH_2N), 2.77 (d, J = 4.8 Hz, 6 H, $N(CH_3)_2$), 2.18-2.24 (m, 2 H, CH_2); ^{13}C NMR [$(CD_3)_2SO$] δ 165.0 (CO_2), 157.3 (C 2), 150.2 (C 4), 131.6 (C 6), 126.0 (C 1), 115.3 (C 5), 108.4 (C 3), 66.5 (CH_2O), 53.7 (CH_2N), 52.6 (OCH_3), 42.0 (N(CH_3)₂), 23.3 (CH_2); Anal. ($C_{13}H_{19}ClN_2O_5$) C, H, N, Cl.

{2-[3-(Dimethylamino)propoxy]-4-nitrophenyl}methanol (**101**). DIBALH (1 M, in DCM, 13.0 mL, 13.0 mmol) was added to a stirred solution of ester **100** (1.05 g, 3.72 mmol) in THF (50 mL) at 5 °C and the solution stirred at 5 °C for 1 h. The solution was poured into a solution of potassium sodium tartrate (1 M, 100 mL) and the mixture stirred vigorously for 20 min. The mixture was extracted with EtOAc (3 × 100 mL), the combined organic fraction washed with water (100 mL), brine (50 mL), dried, and the solvent evaporated. The residue was purified by chromatography on alumina, eluting with a gradient (0-10%) of MeOH/EtOAc, to give **101** (0.81 g, 86%) as a pale yellow solid, mp (EtOAc) 104-105 °C; 1H NMR δ 7.87 (dd, J = 8.3, 2.1 Hz, 1 H, H 5), 7.69 (d, J = 2.1 Hz, 1 H, H 3), 7.64 (d, J = 8.3 Hz, 1 H, H 6), 5.43 (br s, 1 H, OH), 4.58 (s, 2 H, CH_2O), 4.14 (t, J = 6.5 Hz, 2 H, CH_2O), 2.36 (t, J = 7.0 Hz, 2 H, CH_2N), 2.15 (s, 6 H, $N(CH_3)_2$), 1.85-1.91 (m, 2 H, CH_2); ^{13}C NMR δ 155.2 (C 2), 147.0 (C 4), 138.9 (C 1), 126.7 (C 6), 115.3 (C 5),

105.2 (C 3), 66.5 (CH₂O), 57.6 (CH₂O), 55.5 (NCH₂), 45.1 (N(CH₃)₂), 26.5 (CH₂); Anal. (C₁₂H₁₈N₂O₄) C, H, N.

2-[3-(Dimethylamino)propoxy]-4-nitrobenzyl 1-(chloromethyl)-3-[(5,6,7-trimethoxy-
 5 1*H*-indol-2-yl)carbonyl]-2,3-dihydro-1*H*-benzo[*e*]indol-5-ylcarbamate (102). A
 solution of triphosgene (17 mg, 55 μmol) in DCM (2 mL) was added dropwise to a stirred
 solution of amine 1 [G. J. Atwell, W. R. Wilson, W. A. Denny, *Bioorg. Med. Chem. Lett.*,
 1997, 7, 1483] (65 mg, 140 μmol) and Et₃N (44 μL, 313 μmol) in DCM (10 mL) and
 stirred at 20 °C for 2 h. A solution of alcohol 101 (44 mg, 172 μmol) in DCM (2 mL) was
 10 added, followed by nBu₂Sn(OAc)₂ (2 drops) and the solution stirred at 20 °C for 24 h. The
 solvent was evaporated and the residue purified by chromatography, eluting with a gradient
 (0-20%) MeOH/EtOAc, to give 102 (57 mg, 55%) as a yellow solid which was converted
 to the hydrochloride salt, mp (MeOH) 176-180 °C; ¹H NMR [(CD₃)₂SO] δ 11.43 (s, 1 H,
 indole-NH), 10.47 (br s, 1 H, NH⁺Cl⁻), 9.92 (s, 1 H, OCONH), 8.58 (s, 1 H, H 4), 8.10 (d, *J*
 15 = 8.5 Hz, 1 H, H 6), 7.97 (d, *J* = 8.3 Hz, 1 H, H 9), 7.93 (dd, *J* = 8.4, 2.0 Hz, 1 H, H 5''),
 7.82 (d, *J* = 2.0 Hz, 1 H, H 3''), 7.71 (br d, *J* = 8.4 Hz, 1 H, H 6''), 7.56-7.61 (m, 1 H, H 8),
 7.46-7.51 (m, 1 H, H 7), 7.10 (d, *J* = 2.1 Hz, 1 H, H 3'), 6.97 (s, 1 H, H 4'), 5.34 (s, 2 H,
 CH₂O), 4.81 (dd, *J* = 10.8, 9.5 Hz, 1 H, H 2), 4.53 (dd, *J* = 10.8, 1.7 Hz, 1 H, H 2), 4.33-
 4.38 (m, 1 H, H 1), 4.30 (t, *J* = 5.9 Hz, 2 H, CH₂O), 4.07 (dd, *J* = 11.1, 2.9 Hz, 1 H, CH₂Cl),
 20 3.94-3.97 (m, 4 H, CH₂Cl, OCH₃), 3.83 (s, 3 H, OCH₃), 3.81 (s, 3 H, OCH₃), 3.23-3.27 (t, *J*
 = 7.5 Hz, 2 H, CH₂N), 2.75 (s, 6 H, N(CH₃)₂), 2.17-2.23 (m, 2 H, CH₂); ¹³C NMR
 [(CD₃)₂SO] δ 160.2 (CO), 155.8 (C 2''), 154.3 (OCONH), 149.2 (C 5'), 148.0 (C 4''), 141.5
 (C 3a), 139.9 (C 6'), 138.9 (C 7'), 134.3 (C 5), 132.9 (C 1''), 130.7 (C 2'), 129.5 (C 9a),
 128.5 (C 6''), 127.2 (C 8), 125.4 (C 7a'), 124.4 (C 7), 123.7 (C 9), 123.3 (C 6), 123.1 (C
 25 3a'), 122.1 (C 9b), 121.2 (C 5a), 115.7 (C 5''), 113.0 (C 4), 106.3 (C 3', C 3''), 98.0 (C 4'),
 66.8 (CH₂O), 61.0 (OCH₃), 60.9 (OCH₃), 60.7 (CH₂O), 55.9 (OCH₃), 54.9 (C 2), 53.7
 (CH₂N), 47.5 (CH₂Cl), 42.0 (N(CH₃)₂), 41.1 (C 1), 23.6 (CH₂); Anal. (C₃₈H₄₁ClN₅O₉·2HCl):
 C, H, N.

30 **Example 4K. Preparation of 2-[3-(4-morpholinyl)propoxy]-4-nitrobenzyl 1-**
(chloromethyl)-3-[(5,6,7-trimethoxy-1*H*-indol-2-yl)carbonyl]-2,3-dihydro-1*H*-
benzo[*e*]indol-5-ylcarbamate (105).

Methyl 2-[3-(4-morpholinyl)propoxy]-4-nitrobenzoate (103). A mixture of methyl 2-

- 80 -

hydroxy-4-nitrobenzoate (1.0 g, 5.12 mmol) and K_2CO_3 (1.06 g, 7.68 mmol) in DMF (20 mL) was stirred at 20 °C for 30 min. A solution of 4-(3-chloropropyl)morpholine (0.98 g, 7.68 mmol) in DMF (5 mL) was added and the mixture stirred at 100 °C for 6 h. The mixture was cooled to 20 °C and poured into water (300 mL) and extracted with EtOAc (3 × 100 mL). The combined organic fraction was washed with water (2 × 50 mL), brine (50 mL), dried, and the solvent evaporated. The residue was purified by chromatography, eluting with EtOAc, to give **103** (1.33 g, 80%) as an oil, 1H NMR δ 7.88 (d, J = 9.1 Hz, 1 H, H 6), 7.81 (m, 2 H, H 3, H 5), 4.21 (t, J = 6.3 Hz, 2 H, CH_2O), 3.93 (s, 3 H, OCH_3), 3.69-3.74 (m, 4 H, 2 × CH_2O), 2.57 (t, J = 7.0 Hz, 2 H, CH_2N), 2.47-2.51 (m, 4 H, 2 × CH_2N), 2.02-2.07 (m, 2 H, CH_2); ^{13}C NMR δ 165.3 (CO_2), 158.6 (C 2), 150.6 (C 4), 132.0 (C 6), 126.2 (C 1), 114.8 (C 5), 107.8 (C 3), 67.6 (CH_2O), 66.9 (2 × CH_2O), 55.0 (CH_2N), 53.7 (2 × CH_2N), 52.5 (OCH_3), 25.9 (CH_2). Compound **103** was conveniently stored as the hydrochloride salt, mp (EtOAc) 160-163 °C; Anal. ($C_{15}H_{20}N_2O_6 \cdot HCl$) C, H, Cl, N, calc. 7.8, found 9.1%.

15

{2-[3-(4-Morpholinyl)propoxy]-4-nitrophenyl}methanol (104). A solution of ester **103** (1.33 g, 4.10 mmol) in THF (100 mL) was added dropwise to a stirred solution of DIBALH (1 M in DCM, 13.5 mL, 13.5 mmol) at 5 °C and the solution stirred at 5 °C for 1 h. The solution was carefully poured into 1 M HCl (50 mL) and stirred for 10 min. The solution was concentrated under reduced pressure, neutralised and extracted with EtOAc (3 × 100 mL). The combined organic fraction was dried and the solvent evaporated. The residue was purified by chromatography on alumina, eluting with a gradient (0-10%) MeOH/EtOAc, to give **104** (1.07 g, 88%) as a tan solid, mp (EtOAc) 105-106 °C; 1H NMR δ 7.83 (dd, J = 8.2, 2.1 Hz, 1 H, H 5), 7.70 (d, J = 2.1 Hz, 1 H, H 3), 7.46 (d, J = 8.2 Hz, 1 H, H 6), 4.71 (s, 2 H, CH_2O), 4.18 (t, J = 6.0 Hz, 2 H, CH_2O), 3.74-3.77 (m, 4 H, 2 × CH_2O), 2.56 (dd, J = 6.7, 6.5 Hz, 2 H, CH_2N), 2.45-2.49 (m, 4 H, 2 × CH_2N), 2.01-2.06 (m, 2 H, CH_2); ^{13}C NMR δ 156.7 (C 2), 148.2 (C 4), 137.2 (C 1), 128.2 (C 6), 116.1 (C 5), 106.2 (C 3), 67.7 (CH_2O), 66.5 (2 × CH_2O), 60.7 (CH_2O), 56.3 (CH_2N), 53.9 (2 × CH_2N), 25.5 (CH_2); Anal. ($C_{14}H_{20}N_2O_5$) C, H, N.

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2-[3-(4-Morpholinyl)propoxy]-4-nitrobenzyl 1-(chloromethyl)-3-[(5,6,7-trimethoxy-1H-indol-2-yl)carbonyl]-2,3-dihydro-1H-benzo[e]indol-5-ylcarbamate (105). A solution of triphosgene (15.5 mg, 52 μ mol) in DCM (2 mL) was added dropwise to a stirred

solution of amine 1 [G. J. Atwell, W. R. Wilson, W. A. Denny, *Bioorg. Med. Chem. Lett.*, **1997**, 7, 1483] (52 mg, 133 μ mol) and Et₃N (42 μ L, 299 μ mol) in DCM (10 mL) and stirred at °C for 2 h. A solution of alcohol **104** (49 mg, 164 μ mol) in DCM (2 mL) was added, followed by nBu₂Sn(OAc)₂ (2 drops) and the solution stirred at 20 °C for 24 h. The solvent was evaporated and the residue purified by chromatography, eluting with a gradient (0-10%) MeOH/EtOAc, to give **105** (96 mg, 92%) as a tan powder mp (EtOAc) 102-107 °C; ¹H NMR δ 9.48 (s, 1 H, indole-NH), 8.93 (s, 1 H, OCONH), 7.91 (d, *J* = 8.5 Hz, 1 H, H 6), 7.78-7.84 (m, 2 H, H 9, H 5"), 7.73 (d, *J* = 1.8 Hz, 1 H, H 3"), 7.57 (ddd, *J* = 8.2, 7.0, 1.0 Hz, 1 H, H 8), 7.50-7.53 (m, 1 H, H 6"), 7.45 (ddd, *J* = 8.5, 7.0, 1.0 Hz, 1 H, H 7), 7.28 (br s, 1 H, H 4), 7.00 (d, *J* = 2.2 Hz, 1 H, H 3'), 6.88 (s, 1 H, H 4'), 5.36 (s, 2 H, CH₂O), 4.81 (dd, *J* = 10.8, 1.8 Hz, 1 H, H 2), 4.68 (dd, *J* = 10.8, 8.7 Hz, 1 H, H 2), 4.16-4.22 (m, 3 H, CH₂O, CH₂Cl), 4.08 (s, 3 H, OCH₃), 3.93-3.99 (m, 4 H, H 1, OCH₃), 3.92 (s, 3 H, OCH₃), 3.69 (t, *J* = 4.6 Hz, 4 H, 2 \times CH₂O), 3.49 (t, *J* = 10.9 Hz, 1 H, CH₂Cl), 2.55 (t, *J* = 7.1 Hz, 2 H, CH₂N), 2.43-2.49 (m, 4 H, 2 \times CH₂N), 2.00-2.08 (m, 2 H, CH₂); ¹³C NMR δ 160.4 (CO), 156.7 (C 2"), 154.0 (OCONH), 150.2 (C 5'), 148.6 (C 4"), 141.7 (C 3a), 140.6 (C 6'), 138.9 (C 7'), 133.9 (C 5), 132.2 (C 1"), 129.8 (C 2'), 129.6 (C 9a), 128.8 (C 6"), 127.5 (C 8), 125.7 (C 7a'), 125.0 (C 7), 123.6 (C 3a'), 123.2 (C 9), 122.4 (C 6, C 9b), 121.6 (C 5a), 115.7 (C 5"), 112.2 (C 4), 106.5 (C 3'), 106.1 (C 3"), 97.7 (C 4'), 67.1 (CH₂O), 66.8 (2 \times CH₂O), 61.9 (CH₂O), 61.5 (OCH₃), 61.1 (OCH₃), 56.3 (OCH₃), 55.2 (C 2), 54.9 (CH₂N), 53.6 (2 \times CH₂N), 45.8 (CH₂Cl), 43.4 (C 1), 26.1 (CH₂); MS (FAB⁺) *m/z* 788 (MH⁺, 6%), 790 (MH⁺, 3); HRMS (FAB⁺) calc. for C₄₀H₄₃³⁵ClN₅O₁₀ (MH⁺) *m/z* 788.2699, found 788.2721; calc. for C₄₀H₄₃³⁷ClN₅O₁₀ (MH⁺) *m/z* 790.2699, found 790.2728; Anal. (C₄₀H₄₂ClN₅O₁₀·½H₂O) C, H, N.

Example 4L. Preparation of 2-[3-(4-morpholinyl)propoxy]-4-nitrobenzyl bis(3-[(5-methyl-4-acridinyl)carbonyl]amino}propyl)carbamate (107). DIEA (0.3 mL, 3 mmol) was added to a suspension of carbamate dihydrochloride **91** (44 mg, 0.5 mmol) in DCM (10 mL) at 5 °C and the mixture stirred for 10 min. Methanesulfonyl chloride (0.1 mL), was added and the mixture stirred for 30 min. MeOH (2 mL) was added, the mixture stirred for 10 min, and the solvent evaporated. The residue was purified by chromatography on alumina-90, eluting with 1%MeOH/40%EtOAc/DCM to give the crude mesylate **106** (42 mg, 95%) as a yellow solid, ¹H NMR δ 11.94 (s, 1 H, NH), 11.85 (s, 1 H, NH), 8.94 (m, 2 H), 8.77 (m, 2 H), 8.07 (m, 2 H), 7.81 (m, 2 H), 7.62 (m, 4 H), 7.43 (m, 2 H), 7.30 (d, *J* =

- 82 -

2.0 Hz, 1 H, H 3'''), 7.05 (d, $J = 8.4$ Hz, 1 H, H 6'''), 6.95 (dd, $J = 8.4, 2.0$ Hz, 1 H, H 5'''), 5.02 (s, 2 H, CH₂O), 4.39 (t, 2 H, CH₂O), 3.98 (t, 2 H), 3.73-3.58 (m, 8 H), 3.02 (s, 3 H, CH₃SO₂), 2.85 (s, 3 H, CH₃), 2.76 (s, 3 H, CH₃), 2.20-2.05 (m, 6 H).

A solution of mesylate **106** (42 mg, 0.047 mmol) in morpholine (0.5 mL) was stirred at 20 °C for 20 hrs. The reaction mixture was purified by chromatography on alumina-90, eluting with 1%MeOH/40%EtOAc/DCM to give **107** (38 mg, 91%) as a yellow solid, ¹H NMR δ 11.94 (s, 1 H, NH), 11.86 (s, 1H, N H), 8.89-8.91 (m, 2 H), 8.73-8.77 (m, 2 H), 8.06-8.08 (m, 2 H), 7.80-7.84 (m, 2 H), 7.59-7.64 (m, 4 H), 7.42-7.46 (m, 2 H), 7.32 (d, $J = 2.0$ Hz, 1 H, H 3'''), 7.06 (d, $J = 8.4$ Hz, 1 H, H 6'''), 6.95 (dd, $J = 8.4, 2.0$ Hz, 1 H, H 5'''), 5.03 (s, 2 H, CH₂O), 3.92 (t, 2 H), 3.75-3.67 (m, 8 H, 4 × CH₂N), 3.59 (m, 4 H, 2 × CH₂), 2.86 (s, 3 H, CH₃), 2.74 (s, 3 H, CH₃), 2.46 (br s, 6 H, CH₂N), 2.18-2.04 (m, 4 H), 1.93 (br s, 2 H); HRMS (FAB⁺) calc. for (C₅₁H₅₄N₇O₈) (MH⁺) m/z 892.4034, found 892.4055.

Example 4M. Preparation of 4-{2-[(bis(3-[(5-methyl-4-acridinyl)carbonyl]amino}propyl)amino]carbonyl]oxy)methyl]-5-nitrophenoxy}butanoic acid (111**).**

Methyl 2-(4-*tert*-butoxy-4-oxobutoxy)-4-nitrobenzoate (108**).** A mixture of methyl 2-hydroxy-4-nitrobenzoate (**66**) (0.61 g, 3.09 mmol) and K₂CO₃ (0.64 g, 4.64 mmol) in DMF (20 mL) was stirred at 20 °C for 30 min. A solution of *tert*-butyl 4-bromobutanoate (1.04 g, 4.64 mmol) in DMF (5 mL) was added and the mixture stirred at 100 °C for 6 h. The mixture was cooled to 20 °C, poured into water (300 mL) and extracted with EtOAc (3 100 mL). The combined organic fraction was washed with water (2 50 mL), brine (50 mL), dried, and the solvent evaporated. The residue was purified by chromatography, eluting with 20%EtOAc/light petroleum, to give **108** (0.86 g, 82%) as an oil, IR N 2978, 1728, 1532, 1350, 1285, 1250, 1155 cm⁻¹; ¹H NMR δ 7.89 (d, $J = 8.5$ Hz, 1 H, H 6), 7.85 (dd, $J = 8.5, 2.0$ Hz, 1 H, H 5), 7.79 (d, $J = 2.0$ Hz, 1 H, H 3), 4.17 (t, $J = 6.2$ Hz, 2 H, CH₂O), 3.93 (s, 3 H, OCH₃), 2.47 (t, $J = 7.3$ Hz, 2 H, CH₂CO), 2.11-2.16 (m, 2 H, CH₂), 1.49 (s, 9 H, CH₃); ¹³C NMR δ 172.3 (CO₂), 165.4 (CO₂), 158.5 (C 2), 150.7 (C 4), 132.1 (C 6), 126.2 (C 1), 115.0 (C 5), 107.9 (C 3), 80.6 (OC(CH₃)₃), 68.4 (CH₂O), 52.5 (OCH₃), 31.5 (CH₂), 28.1 (OC(CH₃)₃), 24.4 (CH₂); MS m/z 339 (M⁺, 1%), 308 (2), 197 (50), 87 (90), 57 (100); HRMS calc. for C₁₆H₂₁NO₇ (M⁺) m/z 339.1318, found 339.1314.

2-(4-*tert*-Butoxy-4-oxobutoxy)-4-nitrobenzoic acid (109**).** A mixture of ester **108** (0.61 g,

- 83 -

1.80 mmol) and NaOH (1 M, 9 mL, 9 mmol) in MeOH (20 mL) was stirred at 20 °C for 2 h. The mixture was extracted with diethyl ether (20 mL) and the pH adjusted to 4 with 1 M HCl. The mixture was extracted with EtOAc (3 50 mL), the combined organic fraction washed with brine (50 mL), dried and the solvent evaporated. The residue was crystallized
 5 to give **109** as a white solid, mp (EtOAc/light petroleum) 76-77 °C; IR N 1723, 1678, 1532, 1350, 1260, 1154 cm⁻¹; ¹H NMR [(CD₃)₂SO] δ 13.0 (br s, 1 H, CO₂H), 7.80-7.86 (m, 3 H, H 3, H 5, H 6), 4.20 (t, *J* = 6.2 Hz, 2 H, CH₂O), 2.40-2.46 (m, 2 H, CH₂CO), 1.92-1.99 (m, 2 H, CH₂), 1.40 (s, 9 H, CH₃); ¹³C NMR [(CD₃)₂SO] δ 174.0 (CO₂), 171.8 (CO₂), 166.2 (C 2), 157.0 (C 4), 130.9 (C 6), 128.3 (C 1), 115.1 (C 5), 107.9 (C 3), 79.7 (OC(CH₃)₃),
 10 68.0 (CH₂O), 31.0 (CH₂), 27.7 (OC(CH₃)₃), 24.0 (CH₂); Anal. (C₁₅H₁₉NO₇·¼H₂O) C, H, N.

tert-Butyl 4-[2-(hydroxymethyl)-5-nitrophenoxy]butanoate (110). BH₃.DMS (0.28 mL, 2.8 mmol) was added to a stirred solution of acid **109** and trimethyl borate (0.64 mL, 5.66 mmol) in THF (50 mL) and the solution heated at reflux temperature under N₂ for 3 h. The
 15 solution was cooled to 20 °C, MeOH (2 mL) added carefully and the mixture stirred for 5 min. Water (2 mL) was carefully added, the solution stirred for 5 min, 1 M aq. citric acid solution added and the mixture stirred for 30 min. The mixture was extracted with EtOAc (3 60 mL), the combined organic fraction washed with water (50 mL), brine (30 mL), dried, and the solvent evaporated. The residue was purified by chromatography, eluting
 20 with a gradient (30-50%) of EtOAc/light petroleum, to give **110** (0.30 g, 68%) as a white solid, mp (EtOAc/light petroleum) 42-43 °C; IR N 3434, 1726, 1524, 1346, 1248, 1155 cm⁻¹; ¹H NMR δ 7.85 (dd, *J* = 8.2, 2.1 Hz, 1 H, H 5), 7.68 (d, *J* = 2.1 Hz, 1 H, H 3), 7.53 (d, *J* = 8.2 Hz, 1 H, H 6), 4.76 (d, *J* = 4.6 Hz, 2 H, CH₂O), 4.12 (t, *J* = 6.2 Hz, 2 H, CH₂O), 2.54 (br t, *J* = 5.0 Hz, 1 H, OH), 2.45 (t, *J* = 7.2 Hz, 2 H, CH₂CO), 2.11-2.18 (m, 2 H, CH₂), 1.50 (s,
 25 9 H, CH₃); ¹³C NMR δ 172.3 (CO₂), 156.2 (C 2), 148.1 (C 4), 136.8 (C 1), 127.0 (C 6), 116.0 (C 5), 105.7 (C 3), 80.9 (OC(CH₃)₃), 67.7 (CH₂O), 60.6 (CH₂O), 31.9 (CH₂), 28.1 (OC(CH₃)₃), 24.3 (CH₂); MS (CI, NH₃) *m/z* 329 (M⁺+NH₃, 5%), 312 (MH⁺, 2), 273 (100); HRMS (CI, NH₃) calc. for C₁₅H₂₂NO₆ (MH⁺) *m/z* 312.1447, found 312.1448. Anal. (C₁₅H₂₂NO₆) C, H, N.

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4-{2-[({Bis(3-((5-methyl-4-acridinyl)carbonyl)amino}propyl)amino]carbonyl)oxy)methyl]-5-nitrophenoxy}butanoic acid (113). A solution of alcohol **110** (19 mg, 0.06 mmol) and

- 84 -

- DIEA (25 μ L, 140 μ mol) in DCM (2 mL) was added dropwise to a solution of triphosgene (10 mg, 35 μ mol) in DCM (1.5 mL) over 30 minutes at 5 °C and the solution stirred for 1 hr. A solution of *N,N*-bis[3-(5-methylacridine-4-carboxamido)propyl]amine (**111**) [S. A. Gamage, J. A. Spicer, G. J. Atwell, G. J. Finlay, B. C. Baguley, W. A. Denny, *J. Med. Chem.*, **1999**, *42*, 2383-2393] (24 mg, 42 μ mol) and DIEA (4 μ L, 240 μ mol) in DCM (2 mL) was added and the solution stirred at 20 °C for 16 h. The solvent was evaporated and the residue was purified by chromatography on alumina-90, eluting with 1%MeOH/60%EtOAc/DCM to give crude ester **112** (34 mg, 89%) as a yellow foam, ¹H NMR δ 11.90 (s, 1 H, NH), 11.86 (s, 1 H, NH), 8.85-8.95 (m, 2 H), 8.66-8.76 (m, 2 H), 8.02-8.08 (m, 2 H), 7.78-7.87 (m, 2 H), 7.50-7.64 (m, 4 H), 7.38-7.46 (m, 2 H), 7.25 (d, *J* = 2.0 Hz, 1 H, H 3'), 7.01 (d, *J* = 8.3 Hz, 1 H, H 6'), 6.89 (dd, *J* = 8.3, 2.0 Hz, 1 H, H 5'), 4.77 (s, 2 H, CH₂O), 3.84-3.88 (m, 2 H), 3.58-3.73 (m, 8 H), 2.83 (s, 3 H, CH₃), 2.73 (s, 3 H, CH₃), 2.42-3.46 (m, 2 H), 2.10-2.17 (m, 4 H), 2.00-2.02 (m, 2 H), 1.45 (s, 9 H); ¹³C NMR δ 172.2, 166.2, 156.2, 155.6, 155.3, 147.4, 147.0 (2), 145.2 (2), 137.9 (2), 136.9, 135.8, 135.4 (2), 135.2, 132.6, 132.2 (2), 131.0 (2), 127.9 (2), 126.5, 126.4, 126.2, 126.1 (2), 125.8 (2), 125.3, 116.0, 115.1, 105.7, 104.8, 67.7, 61.6, 60.5, 45.9, 45.1, 37.7, 37.0, 31.9, 31.6, 29.2, 28.6, 28.1, 24.3, 24.3, 18.9, 18.8.
- Ester **112** (80 mg, 88 μ mol) was added to a HCl saturated solution of MeOH (20 mL) and DCM (20 mL) at 5 °C and the solution stood for 3 days at 5 °C. The solvent was evaporated and the residue crystallized to give **113** (45 mg, 58%) as a yellow solid, mp (MeOH/EtOAc/light petroleum) 128-130°C; ¹H NMR [(CD₃)₂SO] δ 11.39 (s, 2 H, 2 CONH), 9.15 (s, 1 H), 9.08 (s, 1 H), 8.68 (br s, 2 H), 8.25 (br s, 2 H), 7.95 (br s, 2 H), 7.65 (br s, 4 H), 7.49 (br s, 2 H), 7.32 (d, *J* = 2.0 Hz, 1 H), 7.00 (d, *J* = 8.3 Hz, 1 H), 6.93 (dd, *J* = 8.3, 2.0 Hz, 1 H), 4.77 (s, 2 H, CH₂O), 3.88-3.91 (m, 2 H), 3.50-3.58 (m, 8 H), 2.77 (s, 3 H, CH₃), 2.66 (s, 3 H, CH₃), 2.01 (br s, 4 H), 1.87-1.93 (m, 2 H); Anal. (C₄₈H₄₆N₆O₉·2HCl·2H₂O) C, H, N.

Example 5A. Preparation of (1-methyl-2-nitro-1*H*-imidazol-5-yl)methyl bis(2-chloroethyl)carbamate (117**).**

- 1-Methyl-2-nitro-1*H*-imidazole-5-carboxylic acid (**114**).** Sodium hydroxide solution (1 M, 125 mL, 125 mmol) was added slowly to a stirred suspension of ethyl 1-methyl-2-nitro-1*H*-imidazole-5-carboxylate (**114**) [B. Cavalleri, R. Ballotta, G.C. Lancini. *J. Heterocyclic Chem.* 1972, *9*, 979] (5.0 g, 25.1 mmol) in water (50 mL) and the mixture stirred at 20 °C

until complete dissolution occurred. The pH of the solution was adjusted to 3 with 5 N HCl and the mixture extracted with EtOAc (3 × 100 mL). The combined organic fractions were dried and the solvent evaporated to give **114** (4.29 g, 100%), as white crystals, mp 160-161 °C (lit. (B. Cavalleri, R. Ballotta, V. Arioli; G.C. Lancini, *J. Med. Chem.* 1973, 16, 557)

5 (EtOAc) 161-163 °C); ¹H NMR [(CD₃)₂SO] δ 13.60 (br s, 1 H, CO₂H), 7.37 (s, 1 H, H 4), 4.20 (s, 3 H, NCH₃); ¹³C NMR [(CD₃)₂SO] δ 160.3, 147.2, 133.7, 127.0, 35.0.

(1-methyl-2-nitro-1*H*-imidazol-5-yl)methanol (**115**). A solution of CDI (7.0 g, 43.1 mmol) and **114** was stirred at 20 °C for 30 min and then added to a stirred solution of
10 NaBH₄ (4.07 g, 108 mmol) in EtOH (10 mL) and the mixture stirred at 20 °C for 1 h. 5 M HCl (20 mL) was added carefully and the mixture stirred for 30 min. The solvent was evaporated and the residue purified by chromatography, eluting with EtOAc, to give **115** (2.23 g, 68%) as a white solid, mp 138-140 °C (lit. [B. Cavalleri; R. Ballotta; V. Arioli; G.C. Lancini, *J. Med. Chem.* 1973, 16, 557] 142-144 °C); ¹H NMR [(CD₃)₂SO] δ 7.12 (s, 1
15 H, H 4), 5.49 (br s, 1 H, OH), 4.55 (s, 2 H, CH₂O), 3.92 (s, 3 H, NCH₃); ¹³C NMR [(CD₃)₂SO] δ 145.6, 138.6, 126.5, 52.9, 34.0.

(1-Methyl-2-nitro-1*H*-imidazol-5-yl)methyl 4-nitrophenyl carbonate (**116**). A solution of 4-nitrophenylchloroformate (0.67 g, 3.34 mmol) in THF (5 mL) was added to a stirred
20 solution of alcohol **115** (0.50 g, 3.18 mmol) and pyridine (283 μL, 3.50 mmol) in THF (50 mL) at 20 °C under N₂. The solution was stirred at 20 °C for 16 h, the solvent evaporated and the residue dissolved in EtOAc (100mL). The solution was washed with water (2 × 50 mL), brine (50 mL), dried and the solvent evaporated. The residue was purified by chromatography, eluting with 50% EtOAc/light petroleum, to give **116** (0.87 g, 84%) as a
25 white solid, mp (EtOAc) 156.5-157.5 °C; IR N 1771, 1537, 1359 cm⁻¹; ¹H NMR [(CD₃)₂SO] δ 8.33 (ddd, *J* = 9.1, 3.2, 2.1 Hz, 2 H, H 3, H 5), 7.59 (ddd, *J* = 9.1, 3.2, 2.1 Hz, 2 H, H 2, H 6), 7.37 (s, 1 H, H 4'), 5.48 (s, 2 H, CH₂O), 4.00 (s, 3 H, NCH₃); ¹³C NMR [(CD₃)₂SO] δ 155.1, 151.4, 146.3, 145.2, 131.5, 129.6, 125.4 (2), 122.5 (2), 59.4, 34.3; Anal. (C₁₂H₁₀N₄O₇) C, H, N calc. 17.4, found 16.7%.

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(1-Methyl-2-nitro-1*H*-imidazol-5-yl)methyl bis(2-chloroethyl)carbamate (**117**). A solution of carbonate **116** (0.68 g, 2.11 mmol) in pyridine (3 mL) was added to a solution of bis-(2-chloroethyl)amine hydrochloride (0.75 g, 4.22 mmol) in pyridine (30 mL) under

- 86 -

N₂. The solution was stirred at 20 °C for 16 h and the solvent evaporated. The residue was dissolved in DCM (100 mL) and washed with 2% citric acid solution (2 × 50 mL), water (50 mL), brine (50 mL), dried and the solvent evaporated. The residue was purified by chromatography, eluting with 50% EtOAc/light petroleum, to give **117** (0.51 g, 74%) as
 5 white crystals, mp (EtOAc) 100-101 °C; IR N 1703, 1489, 1344 cm⁻¹; ¹H NMR δ 7.23 (s, 1 H, H 4), 5.21 (s, 2 H, CH₂O), 4.05 (s, 3 H, NCH₃), 3.58-3.70 (m, 8 H, 2 × CH₂N, 2 × CH₂Cl); ¹³C NMR δ 154.8, 144.5, 132.1, 129.7, 56.2, 50.8 (2), 41.6 (2), 34.3; Anal. (C₁₀H₁₄Cl₂N₄O₄) C, H, N.

10 **Example 5B. Preparation of (1-methyl-2-nitro-1*H*-imidazol-5-yl)methyl 4-[bis(2-chloroethyl)amino]phenylcarbamate (123).**

N,N-Bis(2-[[*tert*-butyl(dimethyl)silyl]oxy]ethyl)-4-nitroaniline (**119**). A solution of TBDMSCl (4.20 g, 27.9 mmol) in DMF (15 mL) was added to a stirred solution of *N,N*-bis(2-hydroxyethyl)-4-nitroaniline (**118**) (3.0 g, 13.26 mmol) and imidazole (3.79 g, 55.7
 15 mmol) in DMF (50 mL) and the solution stirred at 20 °C for 48 h. The solvent was evaporated and the residue partitioned between EtOAc (150 mL) and water (150 mL). The organic fraction was washed with water (2 × 200 mL), dried, and the solvent evaporated. The residue was purified by chromatography, eluting with 10% EtOAc/light petroleum, to give **119** (5.72 g, 95%) as a white solid, mp (pet. ether) 48-49 °C; IR N 1597,
 20 1520, 1300, 1202, 1107 cm⁻¹; ¹H NMR δ 8.07 (ddd, *J* = 9.5, 3.5, 2.1 Hz, 2 H, H 3, H 5), 6.67 (ddd, *J* = 9.5, 3.5, 2.1 Hz, 2 H, H 2, H 6), 3.80 (dd, *J* = 6.0, 5.7 Hz, 4 H, 2 × CH₂N), 3.63 (dd, *J* = 5.9, 5.7 Hz, 4 H, 2 × CH₂O), 0.86 (s, 18 H, 2 × SiC(CH₃)₃), 0.01 (s, 12 H, 2 × Si(CH₃)₂); ¹³C NMR δ 153.0, 138.6, 126.2 (2), 110.4 (2), 60.2 (2), 53.6 (2), 25.8 (6), 18.2 (2), -5.5 (4); MS (DEI) *m/z* 454 (M⁺, 10%), 439 (5), 397 (10), 309 (100); HRMS (DEI)
 25 calc. for C₂₂H₄₂N₂O₄Si₂ (M⁺) *m/z* 454.2683, found 454.2668; Anal. (C₂₂H₄₂N₂O₄Si₂) C, H, N.

*N*¹,*N*¹-Bis(2-[[*tert*-butyl(dimethyl)silyl]oxy]ethyl)-1,4-benzenediamine (**120**). A mixture of **119** (1.54 g, 3.39 mmol) and Pd/C (50 mg) in EtOAc/EtOH (1:1) (50 mL) was stirred
 30 under hydrogen (60 psi) for 30 minutes, filtered through celite, washed with EtOH (2 × 10 mL) and the solvent evaporated to give crude benzenediamine (**120**) as an oil that was used directly without further purification or characterization.

(1-Methyl-2-nitro-1*H*-imidazol-5-yl)methyl 4-[bis(2-*tert*-butyl(dimethyl)silyl)oxy]ethyl]amino]phenylcarbamate (**121**). A solution of carbonate **116** (0.87 g, 2.68 mmol), **120** (3.39 mmol), and pyridine (217 μ L, 2.68 mmol) in THF (50 mL) was stirred at 20 °C for 48 h. The solvent was evaporated and the residue partitioned
5 between EtOAc (100 mL) and water (100 mL). The organic fraction was washed with water (2 \times 50 mL), brine (50 mL), dried, and the solvent evaporated. The residue was purified by chromatography, eluting with a gradient (20-50%) of EtOAc/light petroleum to give **121** (1.37 g, 84%) as a white solid, mp 143-144 °C; IR N 3258, 1721, 1539, 1257, 1103 cm^{-1} ; ^1H NMR [$(\text{CD}_3)_2\text{SO}$] δ 7.23 (s, 1 H, H 4'), 7.15 (br d, J = 8.9 Hz, 2 H, H 3, H 5),
10 6.63 (d, J = 8.9 Hz, 2 H, H 2, H 6), 6.52 (br s, 1 H, OCONH), 5.20 (s, 2 H, CH_2O), 4.06 (s, 3 H, NCH_3), 3.73 (dd, J = 6.5, 6.3 Hz, 4 H, 2 \times CH_2O), 3.47 (dd, J = 6.5, 6.3 Hz, 4 H, 2 \times CH_2N), 0.88 (s, 18 H, 2 \times $\text{Si}(\text{C}(\text{CH}_3)_3$), 0.02 (s, 12 H, 2 \times $\text{Si}(\text{CH}_3)_2$); ^{13}C NMR [$(\text{CD}_3)_2\text{SO}$] δ 152.9, 146.1, 145.4, 132.5, 129.6, 125.4, 121.7 (2), 111.8 (2), 60.3 (2), 55.2, 53.6 (2), 34.3, 26.0 (6), 18.2 (2), -5.4 (4); Anal. ($\text{C}_{28}\text{H}_{49}\text{N}_5\text{O}_6\text{Si}_2$) C, H, N.

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(1-Methyl-2-nitro-1*H*-imidazol-5-yl)methyl 4-[bis(2-hydroxyethyl)amino]phenylcarbamate (**122**). TBAF (1 M in THF, 3.9 mL, 3.9 mmol) was added dropwise to a stirred solution of **121** (1.07 g, 1.76 mmol) in THF (30 mL) at 5 °C. The solution was stirred for 30 minutes and the solvent evaporated. The residue was
20 purified by chromatography, eluting with a gradient (0-10%) of MeOH/EtOAc, to give **122** (0.59 g, 88%) as a white solid, mp (MeOH) 171-174 °C; IR N 3445, 3329, 3266, 1717, 1609, 1549, 1491, 1375, 1248 cm^{-1} ; ^1H NMR [$(\text{CD}_3)_2\text{SO}$] δ 9.37 (s, 1 H, OCONH), 7.28 (s, 1 H, H 4'), 7.19 (br d, J = 9.1 Hz, 2 H, H 3, H 5), 6.61 (d, J = 9.1 Hz, 2 H, H 2, H 6), 5.23 (s, 2 H, CH_2O), 4.71 (t, J = 5.4 Hz, 2 H, 2 \times OH), 3.96 (s, 3 H, NCH_3), 3.51 (dd, J = 6.4, 5.9
25 Hz, 4 H, 2 \times CH_2O), 3.34 (dd, J = 6.2, 5.9 Hz, 4 H, 2 \times CH_2N); ^{13}C NMR [$(\text{CD}_3)_2\text{SO}$] δ 152.8, 146.0, 144.2, 133.6, 128.6, 127.1, 120.3 (2), 111.4 (2), 59.7, 58.2 (2), 53.4 (2), 34.2; MS (FAB $^+$) m/z 380 (MH^+ , 10%), 348 (5); HRMS (FAB $^+$) m/z calc. for $\text{C}_{16}\text{H}_{22}\text{N}_5\text{O}_6$ (MH^+) 380.1570, found 380.1579; Anal. ($\text{C}_{16}\text{H}_{21}\text{N}_5\text{O}_6$) C, H, N.

30 (1-Methyl-2-nitro-1*H*-imidazol-5-yl)methyl 4-[bis(2-chloroethyl)amino]phenylcarbamate (**123**). Methanesulfonyl chloride (191 μ L, 2.47 mmol) was added dropwise to a stirred solution of diol **122** (312 mg, 0.82 mmol) in pyridine (10 mL) at 5 °C and the solution stirred at 20 °C for 1 h. The solvent was

- 88 -

evaporated and the residue partitioned between DCM (100 mL) and water (100 mL). The aqueous fraction was washed with DCM (2 × 50 mL), the combined organic extracts dried, and the solvent evaporated. The residue was dissolved in DMF (10 mL), LiCl (210 mg, 4.9 mmol) added and the mixture stirred at 80 °C for 3 h. The solvent was evaporated and the residue partitioned between EtOAc (100 mL) and water (100 mL). The aqueous fraction was extracted with EtOAc (2 × 50 mL), the combined extracts dried, and the solvent evaporated. The residue was purified by chromatography, eluting with 50% EtOAc/light petroleum, to give 123 (227 mg, 66%) as a white solid, mp (MeOH) 156-157 °C; IR N 3408, 3246, 1725, 1531, 1354, 1221 cm⁻¹; ¹H NMR [(CD₃)₂SO] δ 9.49 (s, 1 H, OCONH), 7.26-7.29 (m, 3 H, H 4', H 3, H 5), 6.70 (d, *J* = 9.1 Hz, 2 H, H 2, H 6), 5.24 (s, 2 H, CH₂O), 3.96 (s, 3 H, NCH₃), 3.64-3.70 (m, 8 H, 2 × CH₂N, 2 × CH₂Cl); ¹³C NMR [(CD₃)₂SO] δ 152.8, 146.0, 142.3, 133.5, 128.7, 128.6, 120.3 (2), 112.3 (2), 54.9, 52.2 (2), 41.1 (2), 34.1. Anal. (C₁₆H₁₉Cl₂N₅O₄) C, H, N, Cl.

15 Example 5C. Preparation of (1-methyl-2-nitro-1*H*-imidazol-5-yl)methyl 1-(chloromethyl)-3-[(5,6,7-trimethoxy-1*H*-indol-1-yl)carbonyl]-2,3-dihydro-1*H*-benzo[*e*]indol-5-ylcarbamate (124). A solution of triphosgene (14.5 mg, 49 μmol) in DCM (2 mL) was added dropwise to a stirred solution of amine 1 [G. J. Atwell, W. R. Wilson, W. A. Denny, *Bioorg. Med. Chem. Lett.*, 1997, 7, 1483] (58 mg, 124 μmol) and Et₃N (39 mL, 280 μmol) in DCM (10 mL) and stirred at 20 °C for 2 h. A solution of alcohol 115 (24 mg, 154 μmol) in DCM (2 mL) was added, followed by nBu₂Sn(OAc)₂ (2 drops) and the solution stirred at 20 °C for 48 h. The solvent was evaporated and the residue purified by chromatography, eluting with 40% EtOAc/light petroleum, to give 124 (55 mg, 68%) as a tan powder, mp (EtOAc) 202-204 °C; ¹H NMR δ 9.49 (br s, 1 H, indole-NH), 8.81 (br s, 1 H, H 4), d, *J* = 8.5 Hz, 1 H, H 6), 7.78 (d, *J* = 8.3 Hz, 1 H, H 9), 7.57 (m, 1 H, H 8), 7.43 (m, 1 H, H 7), 7.25 (s, 1 H, H 4''), 7.21 (br s, 1 H, OCONH), 7.00 (d, *J* = 1.6 Hz, 1 H H 3'), 6.87 (s, 1 H, H 4'), 5.31 (d, *J* = 13.6 Hz, 1 H, CH₂O), 5.25 (d, *J* = 13.6 Hz, 1 H, CH₂O), 4.80 (dd, *J* = 10.5, 1.6 Hz, 1 H, H 2), 4.65 (dd, *J* = 10.5, 8.7 Hz, 1 H, H 2), 4.13-4.20 (m, 1 H, H 1), 4.11 (s, 3 H, OCH₃), 4.01 (br s, 3 H, NCH₃), 3.94-3.98 (m, 4 H, CH₂Cl, OCH₃), 3.92 (s, 3 H, OCH₃), 3.47 (dd, *J* = 10.8, 10.8 Hz, 1 H, CH₂Cl); ¹³C NMR δ 160.4 (CO), 153.5 (OCONH), 150.2 (C 5'), 146.4 (C 2''), 141.6 (C 3a), 140.7 (C 6'), 138.9 (C 7'), 133.3 (C 5), 132.1 (C 5''), 129.8 (C 4''), 129.7 (C 9a), 129.5 (C 2'), 127.6 (C 8), 125.7 (C 7a'), 125.1 (C 7, C 5a), 123.6 (C 3a'), 123.2 (C 9), 122.3 (C 6, C 9b), 113.0 (C 4), 106.6

(C 3'), 97.6 (C 4'), 61.5 (OCH₃), 61.2 (OCH₃), 56.3 (OCH₃), 55.8 (CH₂O), 54.9 (C 2), 45.8 (CH₂Cl), 43.4 (C 1), 34.3 (NCH₃); MS (FAB⁺) *m/z* 649 (MH⁺, 2%); HRMS (FAB⁺) calc. for C₃₁H₃₀³⁵ClN₆O₈ (MH⁺) *m/z* 649.1814, found 649.1767; calc. for C₃₁H₃₀³⁷ClN₆O₈ (MH⁺) *m/z* 651.1784, found 651.1819; Anal (C₃₁H₂₉ClN₆O₈·½H₂O) C, H, N.

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Alternative preparation of (1-methyl-2-nitro-1*H*-imidazol-5-yl)methyl 1-(chloromethyl)-3-[(5,6,7-trimethoxy-1*H*-indol-1-yl)carbonyl]-2,3-dihydro-1*H*-benzo[*e*]indol-5-ylcarbamate (124). A solution of alcohol 115 (17 mg, 0.11 mmol) in DCM (2 mL) was added dropwise to a stirred solution of triphosgene (12 mg, 0.04 mmol) and pyridine (9 µL, 0.11 mmol) in DCM (2 mL) at 20 °C. The mixture was stirred at 20 °C for 2 h, the solvent evaporated and the residue dissolved in THF (5 mL). A solution of amine 1 [G. J. Atwell, W. R. Wilson, W. A. Denny, *Bioorg. Med. Chem. Lett.*, **1997**, 7, 1483] (50 mg, 0.11 mmol) in THF (5 mL) was added and the solution stirred at 20 °C for 16 h. The mixture was partitioned between EtOAc (50 mL) and sat. aq. KHCO₃ solution, the organic fraction dried, and the solvent evaporated. The residue was purified by chromatography, eluting with a gradient (50-100%) of EtOAc/light petroleum to give 124 (23 mg, 33%) as a tan solid, mp 200-205 °C (dec); spectroscopically identical with an authentic sample prepared above.

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Example 5D. Preparation of (1-methyl-2-nitro-1*H*-imidazol-5-yl)methyl doxorubicin carbamate (125). A solution of (1-methyl-2-nitro-1*H*-imidazol-5-yl)methyl 4-nitrophenyl carbonate (116) (33 mg, 104 µmol) in DMF (2 mL) was added dropwise to a stirred solution of doxorubicin (13) (46 mg, 86 µmol) and Et₃N (15 µL, 104 µmol) in DMF (5 mL) at 20 °C and the solution stirred for 16 h. The solvent was evaporated and the residue purified by chromatography, eluting with a gradient (0-5%) of MeOH/DCM, to give 125 (44 mg, 70%) as a red solid, mp (DCM) 162-166 °C; ¹H NMR [(CD₃)₂SO] δ 13.96 (s, 1 H, 6-OH), 13.21 (s, 1 H, 11-OH), 7.82-7.87 (m, 2 H, H 1, H 3), 7.58 (dd, *J* = 7.5, 2.1 Hz, 1 H, H 2), 7.18 (s, 1 H, H 4''), 7.02 (d, *J* = 7.9 Hz, 1 H, OCONH), 5.42 (s, 1 H, 9-OH), 5.21 (d, *J* = 2.6 Hz, 1 H, H 1'), 5.07 (s, 2 H, CH₂O), 4.86-4.91 (m, 2 H, H 7, 14-OH), 4.73 (d, *J* = 5.9 Hz, 1 H, 4-OH), 4.58 (d, *J* = 5.9 Hz, 2 H, H 14), 4.13-4.17 (m, 1 H, H 5'), 3.96 (s, 3 H, 4-OCH₃), 3.88 (s, 3 H, NCH₃), 3.66-3.74 (m, 1 H, H 3'), 3.41-3.46 (m, 1 H, H 4'), 2.97 (d, *J* = 18.3 Hz, 1 H, H 10), 2.87 (d, *J* = 18.3 Hz, 1 H, H 10), 2.21 (d, *J* = 14.0 Hz, 1 H, H 8),

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2.17 (dd, $J = 14.0, 5.4$ Hz, 1 H, H 8), 1.84 (dt, $J = 12.8, 3.5$ Hz, 1 H, H 2'), 1.57 (dd, $J = 12.8, 3.8$ Hz, 1 H, H 2'), 1.12 (d, $J = 6.4$ Hz, 3 H, H 6'); ^{13}C NMR $[(\text{CD}_3)_2\text{SO}]$ δ 213.7 (C 13), 186.3 (C 5), 186.2 (C 12), 160.7 (C 4), 156.0 (6), 154.6 (C 11), 154.4 (OCONH), 145.8 (C 2''), 136.1 (C 2), 135.4 (C 12a), 134.5 (C 6a), 134.0 (C 10a), 133.8 (C 5''), 128.3 (C 4''), 119.8 (C 4a), 119.6 (C 1), 118.9 (C 3), 110.6 (C 5a), 110.5 (C 11a), 100.3 (C 1'), 74.9 (C 9), 69.8 (C 7), 67.8 (C 4'), 66.6 (C 5'), 63.7 (C 14), 56.5 (4-OCH₃), 54.7 (CH₂O), 47.3 (C 3'), 38.4 (C 8), 34.1 (NCH₃), 32.0 (C 10), 29.7 (C 2'), 17.0 (C 6'); MS (FAB⁺) m/z 727 (MH⁺, 0.2%); HRMS (FAB⁺) calc. for C₃₃H₃₄N₄O₁₅ (MH⁺) m/z 727.2099, found 727.2075; Anal (C₃₃H₃₄N₄O₁₅·½H₂O) C, H, N.

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Example 5E. Preparation of 4-(((1-methyl-2-nitro-1*H*-imidazol-5-yl)methoxy)carbonyl)amino)benzyl doxorubicin carbamate (129).

(1-Methyl-2-nitro-1*H*-imidazol-5-yl)methyl 4-((*tert*-

butyl(dimethyl)silyl]oxy)methyl)phenylcarbamate (126). Et₃N (0.26 mL, 1.86 mmol)

15 was added to a stirred suspension of (1-methyl-2-nitro-1*H*-imidazol-5-yl)methyl 4-

nitrophenyl carbonate (116) (0.50 g, 1.55 mmol), 4-((*tert*-

butyl(dimethyl)silyl]oxy)methyl)aniline (9) (0.40 g, 1.71 mmol), HOBT (0.21 g, 1.55

mmol), and 4 Å molecular sieves (500 mg) in THF (80 mL) and the mixture stirred at 20

°C for 16 h. The solvent was evaporated and the residue partitioned between EtOAc (100

20 mL) and water (100 mL). The organic fraction was washed with 1 M HCl (2 × 40 mL),

water (100 mL), brine (50 mL), dried, and the solvent evaporated. The residue was purified

by chromatography, eluting with 40% EtOAc/light petroleum, to give 126 (0.43 mg, 66%)

as a white solid, mp (EtOAc/light petroleum) 131-132 °C; ^1H NMR δ 7.33 (br d, $J = 8.8$

Hz, 2 H, H 3, H 5), 7.27 (d, $J = 8.8$ Hz, 2 H, H 2, H 6), 7.23 (s, 1 H, H 4'), 6.83 (br s, 1 H,

25 OCONH), 5.22 (s, 2 H, CH₂O), 4.69 (s, 2 H, CH₂O), 4.05 (s, 3 H, NCH₃), 0.93 (s, 9 H,

SiC(CH₃)₃), 0.09 (s, 6 H, Si(CH₃)₂); ^{13}C NMR δ 152.3 (OCONH), 146.1 (C 2'), 137.4 (C 1),

135.8 (C 4), 132.5 (C 5'), 129.6 (C 4'), 126.9 (C 2, C 6), 118.8 (C 3, C 5), 64.5 (CH₂O),

55.4 (CH₂O), 34.3 (NCH₃), 25.9 (SiC(CH₃)₃), 18.4 (Si(CH₃)₃), -5.3 (Si(CH₃)₂); Anal.

(C₁₉H₂₈N₄O₅Si) C, H, N.

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(1-Methyl-2-nitro-1*H*-imidazol-5-yl)methyl 4-(hydroxymethyl)phenylcarbamate (127).

1 M HCl (2 mL, 2 mmol) was added to a stirred solution of silyl ether 126 (0.39 g, 0.93

mmol) in MeOH (10 mL) and stirred at 20 °C for 1 h. The solution was poured into brine

(50 mL) and extracted with EtOAc (3 × 50 mL). The combined organic fraction was washed with water (50 mL), dried, and the solvent evaporated. The residue was purified by chromatography, eluting with a gradient (50-100%) EtOAc/light petroleum, to give **127** (247 mg, 87%) as a pale yellow solid, mp (EtOAc) 180-181 °C; ¹H NMR [(CD₃)₂SO] δ 9.77 (br s, 1 H, OCONH), 7.40 (d, *J* = 8.5 Hz, 2 H, H 3, H 5), 7.31 (s, 1 H, H 4'), 7.22 (d, *J* = 8.5 Hz, 2 H, H 2, H 6), 5.27 (s, 2 H, CH₂O), 5.08 (t, *J* = 5.6 Hz, 1 H, OH), 4.42 (d, *J* = 5.6 Hz, 2 H, CH₂O), 3.97 (s, 3 H, NCH₃); ¹³C NMR [(CD₃)₂SO] δ 152.6 (OCONH), 146.0 (C 2'), 137.2 (C 1), 136.8 (C 4), 133.3 (C 5'), 128.7 (C 4'), 127.0 (C 2, C 6), 118.0 (C 3, C 5), 62.4 (CH₂O), 55.0 (CH₂O), 34.2 (NCH₃); Anal. (C₁₃H₁₄N₄O₅) C, H, N.

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4-(((1-methyl-2-nitro-1*H*-imidazol-5-yl)methoxy)carbonyl)amino)benzyl 4-nitrophenyl carbonate (128). A solution of 4-nitrophenylchloroformate (216 mg, 1.07 mmol) in THF (5 mL) was added dropwise to a stirred solution of alcohol **127** (219 mg, 0.72 mmol) in THF (40 mL) and the solution stirred at 20 °C for 96 h. The solvent was evaporated and the residue partitioned between EtOAc (100 mL) and water (100 mL). The organic fraction was washed with water (2 × 50 mL), brine (50 mL), dried, and the solvent evaporated. The residue was purified by chromatography, eluting with 50% EtOAc/light petroleum, to give **128** (62 mg, 18%) as a white solid; ¹H NMR [(CD₃)₂SO] δ 9.96 (s, 1 H, OCONH), 8.31 (ddd *J* = 9.2, 3.3, 2.2 Hz, 2 H, H 3'', H 5''), 7.56 (ddd, *J* = 9.2, 3.3, 2.2 Hz, 2 H, H 2'', H 6''), 7.51 (d, *J* = 8.6 Hz, 2 H, H 3, H 5), 7.40 (d, *J* = 8.6 Hz, 2 H, H 2, H 6), 7.31 (s, 1 H, H 4'), 5.33 (s, 2 H, CH₂O), 5.24 (s, 2 H, CH₂O), 3.98 (s, 3 H, NCH₃); ¹³C NMR [(CD₃)₂SO] δ 155.2 (OCO2), 152.6 (OCONH), 151.9 (C 1''), 145.1 (C 2'), 139.2 (C 1), 133.2 (C 4), 129.6 (C 3'', C 5''), 128.8 (C 4'), 128.7 (C 5'), 125.6 (C 2, C 6), 122.2 (C 2'', C 6''), 118.8 (C 2, C 6), 70.2 (CH₂O), 55.2 (CH₂O), 34.2 (NCH₃); MS (FAB⁺) *m/z* 472 (MH⁺, 1%), 443 (0.5); HRMS (FAB⁺) calc. for C₂₀H₁₈N₅O₉ (MH⁺) *m/z* 472.1105, found 472.1106.

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4-(((1-Methyl-2-nitro-1*H*-imidazol-5-yl)methoxy)carbonyl)amino)benzyl doxorubicin carbamate (129). A solution of carbonate **128** (81 mg, 172 μmol) in DMF (2 mL) was added dropwise to a stirred solution of doxorubicin (45 mg, 86 μmol) and Et₃N (15 μL, 103 μmol) in DMF (5 mL) at 20 °C and the solution stirred for 24 h. The solvent was evaporated and the residue purified by chromatography, eluting with 5% MeOH/DCM, to give **129** (57 mg, 75%) as a red solid, mp (DCM) 160-162 °C; ¹H NMR [(CD₃)₂SO] δ

- 92 -

13.99 (s, 1 H, 6-OH), 13.24 (s, 1 H, 11-OH), 9.82 (s, 1 H, OCONH), 7.84-7.89 (m, 2 H, H 1, H 2), 7.60-7.63 (m, 1 H, H 3), 7.40 (d, $J = 8.3$ Hz, 2 H, H 3'', H 5''), 7.29 (s, 1 H, H 4'''), 7.23 (d, $J = 8.3$ Hz, 2 H, H 2'', H 6''), 6.81 (d, $J = 8.0$ Hz, 1 H, OCONH), 5.43 (s, 1 H, H 7), 5.25 (s, 2 H, CH₂O), 5.21 (d, $J = 2.9$ Hz, 1 H, H 1'), 4.89-4.91 (m, 1 H, 9-OH), 4.87 (s, 2 H, CH₂O), 4.84 (dd, $J = 6.3, 5.8$ Hz, 1 H, 14-OH), 4.69 (d, $J = 5.7$ Hz, 1 H, 4-OH), 4.58 (d, $J = 6.0$ Hz, 2 H, H 14), 4.13-4.17 (m, 1 H, H 5'), 3.97 (s, 3 H, OCH₃), 3.95 (s, 3 H, NCH₃), 3.68-3.75 (m, 1 H, H 3'), 3.43-3.46 (m, 1 H, H 4'), 2.99 (d, $J = 18.3$ Hz, 1 H, H 10), 2.91 (d, $J = 18.3$ Hz, 1 H, H 10), 2.21 (br d, $J = 14.1$ Hz, 1 H, H 8), 2.10 (dd, $J = 14.1$ Hz, 1 H, H 8), 1.84 (dt, $J = 12.9, 3.6$ Hz, 1 H, H 2'), 1.47 (dd, $J = 12.9, 3.8$ Hz, 1 H, H 2'), 1.13 (d, $J = 6.5$ Hz, 3 H, H 6'); ¹³C NMR [(CD₃)₂SO] δ 213.7 (C 13), 186.4 (C 5), 186.3 (C 12), 160.7 (C 4), 156.0 (C 6), 155.2 (C 11), 154.4 (OCONH), 152.6 (OCONH), 146.0 (C 2'''), 138.2 (C 4''), 136.1 (C 2), 135.4 (C 12a), 134.6 (C 6a), 134.0 (C 10a), 13.2 (C 5'''), 131.2 (C 1''), 128.7 (C 4'''), 128.6 (C 2'', C 6''), 119.9 (C 4a), 119.6 (C 1), 118.9 (C 3), 118.0 (C 3'', C 5''), 110.7 (C 5a), 110.5 (C 11a), 100.2 (C 1'), 74.9 (C 9), 69.8 (C 7), 67.9 (C 4'), 66.6 (C 5'), 64.8 (C 14), 63.6 (CH₂O), 56.5 (OCH₃), 55.1 (CH₂O), 47.0 (C 3'), 36.5 (C 8), 34.1 (NCH₃), 32.0 (C 10), 29.8 (C 2'), 16.9 (C 6'); MS (FAB⁺) m/z 876 (MH⁺, 0.2%); Anal. (C₄₁H₄₁N₅O₁₇·H₂O) C, H, N.

Example 5F. Preparation of (1-methyl-4-nitro-1H-imidazol-5-yl)methyl 1-

(chloromethyl)-3-[(5,6,7-trimethoxy-1H-indol-1-yl)carbonyl]-2,3-dihydro-1H-benzo[e]indol-5-ylcarbamate (132).

(1-Methyl-4-nitro-1H-imidazol-5-yl)methanol (131). Ozone was bubbled through a solution of 1-methyl-4-nitro-5-[(E)-2-phenylethenyl]-1H-imidazole (130) [D. C. Baker, S. R. Putt, H. D. H. Showalter, *J. Heterocyclic Chem.*, **1983**, *30*, 629-634.] (1.0 g, 4.36 mmol) in DCM/MeOH (1:1, 120 mL) at -78 °C until a blue colour persisted. The solution was warmed to -40 °C with a nitrogen purge to remove excess ozone. A solution of NaBH₄ (165 mg, 4.36 mmol) in EtOH (10 mL) was added dropwise over 15 min and the mixture stirred for 30 min. Acetic acid (0.5 mL) was added and the solvent evaporated. The residue was partitioned between water (50 mL) and light petroleum (50 mL). The aqueous fraction was evaporated and the residue triturated with hot acetone (60 mL). The mixture was filtered and the solution concentrated to give 131 (523 mg, 78%) as a tan powder, mp (acetone) 135-137 °C; ¹H NMR δ 7.78 (s, 1 H, H 2), 5.48 (t, $J = 5.6$ Hz, 1 H, OH), 4.85 (d, $J = 5.6$ Hz, 2 H, CH₂O), 3.75 (s, 3 H, NCH₃); ¹³C NMR δ 143.5 (C 4), 136.8 (C 2), 133.2 (C

- 93 -

5), 51.4 (CH₂O), 32.5 (NCH₃); Anal. (C₃₁H₂₉N₆O₈) C, H, N.

(1-Methyl-4-nitro-1*H*-imidazol-5-yl)methyl 1-(chloromethyl)-3-[(5,6,7-trimethoxy-1*H*-indol-1-yl)carbonyl]-2,3-dihydro-1*H*-benzo[*e*]indol-5-ylcarbamate (132). A solution of
 5 triphosgene (13 mg, 43 μ mol) in DCM (2 mL) was added dropwise to a stirred solution of amine 1 [G. J. Atwell, W. R. Wilson, W. A. Denny, *Bioorg. Med. Chem. Lett.*, 1997, 7, 1483] (51 mg, 109 μ mol) and Et₃N (34 μ L, 246 μ mol) in DCM (10 mL) and stirred at 20 °C for 2 h. A solution of (1-methyl-4-nitro-1*H*-imidazol-5-yl)methanol 131 (23 mg, 147 μ mol) in DCM (2 mL) was added, followed by nBu₂Sn(OAc)₂ (2 drops) and the solution
 10 stirred at 20 °C for 24 h. The solvent was evaporated and the residue purified by chromatography, eluting with EtOAc, to give 132 (40 mg, 56%) as a white powder, mp (EtOAc/light petroleum) 229-231 °C; ¹H NMR δ 9.48 (s, 1 H, indole-NH), 8.87 (s, 1 H, OCONH), 7.87 (d, *J* = 8.5 Hz, 1 H, H 6), 7.76 (d, *J* = 8.3 Hz, 1 H, H 9), 7.55 (ddd, *J* = 8.3, 7.4, 0.7 Hz, 1 H, H 8), 7.44 (ddd, *J* = 8.5, 7.4, 0.7 Hz, 1 H, H 7), 7.40 (s, 1 H, H 2''), 7.33
 15 (br s, 1 H, H 4), 7.00 (d, *J* = 2.3 Hz, 1 H, H 3'), 6.87 (s, 1 H, H 4'), 5.61 (s, 2 H, CH₂O), 4.78 (dd, *J* = 10.7, 1.7 Hz, 1 H, H 2), 4.65 (dd, *J* = 10.7, 8.7 Hz, 1 H, H 2), 4.15-4.20 (m, 1 H, H 1), 4.05-4.10 (m, 4 H, OCH₃, CH₂Cl), 3.95 (s, 3 H, OCH₃), 3.92 (s, 3 H, OCH₃), 3.83 (br s, 3 H, NCH₃), 3.44 (dd, *J* = 10.9, 10.7 Hz, 1 H, CH₂Cl); ¹³C NMR δ 160.4 (CO), 153.8 (OCONH), 150.2 (C 5'), 146.3 (C 4''), 141.5 (C 3a), 140.6 (C 6'), 138.8 (C 7'), 136.3 (C 2''),
 20 133.4 (C 5), 129.7 (C 9a), 129.5 (C 2'), 127.6 (C 8), 126.9 (C 5''), 125.7 (C 7a'), 125.2 (C 7), 125.2 (C 5a), 123.6 (C 3a'), 123.1 (C 9), 122.3 (C 6), 121.9 (C 9b), 112.5 (C 4), 106.6 (C 3'), 97.7 (C 4'), 61.5 (OCH₃), 61.1 (OCH₃), 56.3 (OCH₃), 54.9 (C 2), 54.4 (CH₂O), 45.8 (CH₂Cl), 43.4 (C 1), 33.2 (NCH₃); MS (FAB⁺) *m/z* 651 (MH⁺, 1%), 649 (MH⁺, 2); HRMS (FAB⁺) calc. for C₃₁H₃₀³⁵ClN₆O₈ (MH⁺) *m/z* 649.1814, found 649.1818; calc. for C₃₁H₃₀³⁷ClN₆O₈ (MH⁺) *m/z* 651.1784, found 651.1805; Anal. (C₃₁H₂₉ClN₆O₈) C, H, N.

Example 5G. Preparation of (1-methyl-5-nitro-1*H*-imidazol-2-yl)methyl bis(2-chloroethyl)carbamate (135).

(1-Methyl-5-nitro-1*H*-imidazol-2-yl)methanol (133). A mixture of 1-methyl-5-nitro-1*H*-imidazole [C.E. Hazeldine, F.L. Pyman, *J. Winchester. J. Chem. Soc.* 1924, 1431] (1.0 g,
 30 7.9 mmol) and paraformaldehyde (1.4 g, 15.7 mmol) in DMSO (10 mL) was heated in a sealed tube at 100 °C for 24 h. The mixture was cooled to 20 °C, EtOH (50 mL) was added, and the suspension was filtered. The filtrate was concentrated and the residue was purified

- 94 -

by chromatography on alumina, eluting with a gradient of MeOH/CHCl₃ (0-10%), to give **133** (0.71 g, 57%) as a white solid, mp (CHCl₃) 116-118 °C (lit. [C. Rufer, H. J. Kessler, E. Schroder. *J. Med. Chem.* **1971**, *14*, 94] mp 111 °C); ¹H NMR [(CD₃)₂SO] δ 7.90 (s, 1 H, H 4), 5.62 (t, *J* = 5.8 Hz, 1 H, OH), 4.62 (d, *J* = 5.8 Hz, 2 H, CH₂O), 3.99 (s, 3 H, NCH₃); ¹³C NMR [(CD₃)₂SO] δ 151.8, 138.8, 130.9, 56.1, 32.9.

(1-Methyl-5-nitro-1*H*-imidazol-2-yl)methyl 4-nitrophenyl carbonate (134). A solution of 4-nitrophenyl chloroformate (1.48 g, 7.4 mmol) in THF (8 mL) was added slowly to a stirred solution of alcohol **133** (1.10 g, 7.0 mmol) and pyridine (0.62 mL, 7.7 mmol) in THF (50 mL) at 20 °C under N₂. The mixture was stirred at 20 °C for 16 h, then partitioned between EtOAc/H₂O. The organic fraction was washed with saturated aqueous NaHCO₃ (50 mL), and the solvent evaporated to give **134** (2.04g, 94%) as a tan solid, mp (EtOAc/light petroleum) 168-171 °C; ¹H NMR [(CD₃)₂SO] δ 8.33 (ddd, *J* = 9.1, 3.2, 2.2 Hz, 2 H, H 3, H 5), 8.11 (s, 1 H, H 4'), 7.59 (ddd, *J* = 9.1, 3.2, 2.2 Hz, 2 H, H 2, H 6), 5.48 (s, 2 H, CH₂O), 3.97 (s, 3 H, NCH₃); ¹³C [(CD₃)₂SO] δ 155.0, 151.3, 145.9, 145.2, 139.5, 131.6, 125.3 (2), 122.4 (2), 61.7, 33.5.

(1-Methyl-5-nitro-1*H*-imidazol-2-yl)methyl bis(2-chloroethyl)carbamate (135). A solution of carbonate **134** (2.0 g, 6.5 mmol) in pyridine (5 mL) was added to a stirred solution of *N,N*-bis-(2-chloroethyl)amine hydrochloride (1.5 g, 8.4 mmol) in pyridine (30 mL) at 0 °C. The solution was stirred at 20 °C for 16 h, then solvent was evaporated and the residue was partitioned between DCM (100 mL) and 10% aqueous citric acid (100 mL). The organic fraction was dried, the solvent evaporated, and the residue was purified by chromatography, eluting with 50% EtOAc/light petroleum, to give **135** (2.0 g, 95%) as an oil; ¹H NMR δ 7.99 (s, 1 H, H 4') 5.27 (s, 2 H, CH₂O), 4.03 (s, 3 H, NCH₃), 3.60-3.71 (m, 8 H, 2 × CH₂N, 2 × CH₂Cl); ¹³C NMR δ 154.8, 146.9, 139.6, 132.2, 58.7, 51.1 (2), 41.8 (2), 39.7; HRMS calc. for C₁₀H₁₄Cl₂N₄O₄ (M⁺) *m/z* 324.1392; found 324.1381.

Example 5H. Preparation of (1-methyl-5-nitro-1*H*-imidazol-2-yl)methyl 4-[bis(2-chloroethyl)amino]phenylcarbamate (137). Diphosgene (85mL, 0.7 mmol) was added dropwise to a stirred solution of (1-methyl-5-nitro-1*H*-imidazol-2-yl)methanol (**133**) (0.2 g, 1.27 mmol) and Et₃N (98 mL, 0.7 mmol) in THF (10 mL) at 5 °C. The suspension was stirred at 5 °C for 30 min and a mixture of *N,N'*-bis(2-chloroethyl)-1,4-benzenediamine

- 95 -

hydrochloride (136) (J. L. Everett, W. C. J. Ross. *J. Chem. Soc.* **1949**, 1972] (0.38 g, 1.40 mmol) and Et₃N (195 mL, 1.40 mmol) in THF (4 mL) was added dropwise to the above suspension. The mixture was stirred at 20 °C for 4 h, the solvent evaporated and the residue purified by chromatography, eluting with 50% EtOAc/light petroleum, to give 137 (0.19 g, 36%), mp (CHCl₃/pet. ether) 164-164.5 °C; IR ν 3250, 3185, 3127, 1723, 1603, 1547, 1516, 1381 cm⁻¹; ¹H NMR [(CD₃)₂SO] δ 9.57 (br s, 1 H, OCONH), 8.08 (s, 1 H, H 4'), 7.27 (br d, *J* = 8.0 Hz, 2 H, H 3, H 5), 6.70 (d, *J* = 9.1 Hz, 2 H, H 2, H 6), 5.26 (s, 2 H, CH₂O), 3.95 (s, 3 H, NCH₃), 3.65-3.72 (m, 8 H, 2 × CH₂N, 2 × CH₂Cl); ¹³C NMR [(CD₃)₂SO] δ 152.7, 148.0, 142.3, 139.3, 131.7, 128.7, 120.3 (2), 112.3 (2), 57.4, 52.2 (2), 41.1 (2), 33.4; MS (DEI) *m/z* 415 (M⁺, 1%), 366 (2), 316 (2), 258 (20), 211 (30), 209 (100); HRMS (DEI) *m/z* calc. for C₁₆H₁₉³⁵Cl₂N₅O₄ (M⁺) 415.0814, found 415.0808; calc. for C₁₆H₁₉³⁵Cl³⁷ClN₅O₄ (M⁺) 417.0785, found 417.0781; calc. for C₁₆H₁₉³⁷Cl₂N₅O₄ (M⁺) 419.0755, found 419.0769; Anal. (C₁₆H₁₉Cl₂N₅O₄) C, H, N.

Example SI. Preparation of (1-methyl-5-nitro-1*H*-imidazol-2-yl)methyl 1-(chloromethyl)-3-[(5,6,7-trimethoxy-1*H*-indol-2-yl)carbonyl]-2,3-dihydro-1*H*-benzo[*e*]indol-5-ylcarbamate (138). A solution of triphosgene (12 mg, 41 μ mol) in DCM (2 mL) was added dropwise to a stirred solution of amine 1 [G. J. Atwell, W. R. Wilson, W. A. Denny, *Bioorg. Med. Chem. Lett.*, **1997**, 7, 1483] (48 mg, 103 μ mol) and Et₃N (32 μ L, 231 μ mol) in DCM (10 mL) and stirred at 20 °C for 2 h. A solution of (1-methyl-5-nitro-1*H*-imidazol-2-yl)methanol (133) (20 mg, 127 μ mol) in DCM (2 mL) was added, followed by nBu₂Sn(OAc)₂ (2 drops) and the solution stirred at 20 °C for 24 h. The solvent was evaporated and the residue purified by chromatography, eluting with 40% EtOAc/DCM, to give 138 (23 mg, 34%) as a tan powder mp (EtOAc) 169-170 °C; ¹H NMR δ 9.47 (s, 1 H, indole-NH), 8.85 (s, 1 H, OCONH), 7.98 (s, 1 H, H 4''), 7.86 (d, *J* = 8.5 Hz, 1 H, H 6), 7.77 (d, *J* = 8.3 Hz, 1 H, H 9), 7.57 (br dd, *J* = 8.3, 7.4 Hz, 1 H, H 8), 7.44 (ddd, *J* = 8.5, 7.4, 0.7 Hz, 1 H, H 7), 7.37 (br s, 1 H, H 4), 6.99 (d, *J* = 2.3 Hz, 1 H, H 3'), 6.87 (s, 1 H, H 4'), 5.38 (d, *J* = 13.4 Hz, 1 H, CH₂O), 5.34 (d, *J* = 13.4 Hz, 1 H, CH₂O), 4.79 (dd, *J* = 10.7, 1.6 Hz, 1 H, H 2), 4.65 (dd, *J* = 10.7, 8.7 Hz, 1 H, H 2), 4.15-4.19 (m, 1 H, H 1), 4.09 (s, 3 H, OCH₃), 4.02 (br s, 3 H, NCH₃), 3.95 (s, 3 H, OCH₃), 3.92-3.94 (m, 4 H, OCH₃, CH₂Cl), 3.45 (dd, *J* = 10.9, 10.7 Hz, 1 H, CH₂Cl); ¹³C NMR δ 160.4 (CO), 153.4 (OCONH), 150.2 (C 5'), 146.9 (C 5''), 141.6 (C 3a), 140.7 (C 6'), 139.6 (C 2''), 138.9 (C 7'), 133.3 (C 5), 132.1 (C 4''), 129.7 (C 9a), 129.6 (C 2'), 127.6 (C 8), 125.7 (C 7a'), 125.2 (C 7 and C 5a), 123.6 (C 3a'),

- 96 -

123.2 (C 9), 122.3 (C 6), 122.2 (C 9b), 112.8 (C 4), 106.6 (C 3'), 97.7 (C 4'), 61.5 (OCH₃), 61.2 (OCH₃), 58.4 (CH₂O), 56.3 (OCH₃), 54.9 (C 2), 45.8 (CH₂Cl), 43.4 (C 1), 33.8 (NCH₃); MS (FAB⁺) *m/z* 649 (MH⁺, 3%), 651 (1.5); HRMS (FAB⁺) calc. for C₃₁H₃₀³⁵ClN₆O₈ (MH⁺) *m/z* 649.1814, found 649.1797; calc. for C₃₁H₃₀³⁷ClN₆O₈ (MH⁺) *m/z* 651.1784, found 651.1802; Anal. (C₃₁H₂₉ClN₆O₈) C, H, N.

Example 5J. Preparation of 4-(((1-methyl-5-nitro-1*H*-imidazol-2-yl)methoxy)carbonyl)amino)benzyl doxorubicin carbamate (142).

1-Methyl-5-nitro-1*H*-imidazol-2-yl 4-(((*tert*-

10 butyl(dimethyl)silyl]oxy)methyl)phenylcarbamate (139). Et₃N (1.10 mL, 7.87 mmol) was added to a stirred suspension of (1-methyl-5-nitro-1*H*-imidazol-2-yl)methyl 4-nitrophenyl carbonate (134) (2.31 g, 7.17 mmol), 4-(((*tert*-butyl(dimethyl)silyl]oxy)methyl)aniline (120) (1.79 g, 7.87 mmol), HOBT (0.97 g, 7.17 mmol), and 4 Å molecular sieves (2.5 g) in THF (100 mL) and the mixture stirred at 20 °C

15 for 48 h. The solvent was evaporated and the residue partitioned between EtOAc (100 mL) and water (100 mL). The organic fraction was washed with 1 M HCl (2 × 40 mL), water (100 mL), brine (50 mL), dried, and the solvent evaporated. The residue was purified by chromatography, eluting with 50% EtOAc/light petroleum, to give 139 (2.57 g, 85%) as a white solid, mp (EtOAc/light petroleum) 145-146 °C; ¹H NMR δ 7.99 (s, 1 H, H 4'), 7.32

20 (br d, *J* = 8.1 Hz, 2 H, H 3, H 5), 7.27 (d, *J* = 8.1 Hz, 2 H, H 2, H 6), 6.96 (br s, 1 H, OCONH), 5.30 (s, 2 H, CH₂O), 4.69 (s, 2 H, CH₂O), 4.05 (s, 3 H, NCH₃), 0.93 (s, 9 H, SiC(CH₃)₃), 0.09 (s, 6 H, Si(CH₃)₂); ¹³C NMR δ 152.3 (OCONH), 147.0 (C 5'), 139.6 (C 2'), 137.4 (C 1), 135.8 (C 4'), 129.6 (C 4), 126.9 (C 2, C 6), 118.8 (C 3, C 5), 64.5 (CH₂O), 58.0 (CH₂O), 33.7 (NCH₃), 25.9 (SiC(CH₃)₃), 18.4 (Si(CH₃)₃), -5.3 (Si(CH₃)₂); Anal.

25 (C₁₉H₂₈N₄O₅Si) C, H, N.

(1-Methyl-5-nitro-1*H*-imidazol-2-yl)methyl 4-(hydroxymethyl)phenylcarbamate (140).

1 M HCl (16 mL, 16 mmol) was added to a stirred solution of silyl ether 139 (1.36 g, 3.22 mmol) in MeOH (50 mL) and stirred at 20 °C for 1 h. The solution was poured into brine

30 (50 mL) and extracted with EtOAc (3 × 50 mL). The combined organic fraction was washed with water (50 mL), dried, and the solvent evaporated. The residue was crystallized from EtOAc/light petroleum, to give 140 (0.86g, 47%) as a white solid, mp (EtOAc/light petroleum) 181-183 °C; ¹H NMR [(CD₃)₂SO] δ 9.85 (br s, 1 H, OCONH), 8.09 (s, 1 H, H

- 97 -

4'), 7.40 (d, $J = 8.5$ Hz, 2 H, H 3, H 5), 7.22 (d, $J = 8.5$ Hz, 2 H, H 2, H 6), 5.29 (s, 2 H, CH₂O), 4.42 (s, 2 H, CH₂O), 3.96 (s, 3 H, NCH₃), 3.79 (br s, 1 H, OH); ¹³C NMR [(CD₃)₂SO] δ 152.5 (OCONH), 147.8 (C 5'), 139.3 (C 2'), 137.4 (C 1), 136.8 (C 4), 131.7 (C 2, C 6), 127.0 (C 3, C 5), 118.0 (C 4), 62.4 (CH₂O), 57.5 (CH₂O), 33.4 (NCH₃); Anal.

5 (C₁₃H₁₄N₄O₅) C, H, N.

4-(((1-Methyl-5-nitro-1H-imidazol-2-yl)methoxy)carbonyl)amino)benzyl 4-nitrophenyl carbonate (141). A solution of 4-nitrophenylchloroformate (0.72 g, 3.55 mmol) in THF (10 mL) was added dropwise to a stirred solution of alcohol **140** (0.73 g, 2.37 mmol) and Et₃N (0.66 mL, 4.73 mmol) in THF (40 mL) and the solution stirred at 20 °C for 16 h. The solvent was evaporated and the residue was purified by chromatography, eluting with 20% EtOAc/DCM, to give **141** (0.71 g, 63%) as a white solid; ¹H NMR [(CD₃)₂SO] δ 10.02 (s, 1 H, OCONH), 8.31 (ddd $J = 9.1, 3.3, 2.1$ Hz, 2 H, H 3, H 5), 8.08 (s, 1 H, H 4'), 7.57 (ddd, $J = 9.1, 3.3, 2.1$ Hz, 2 H, H 2, H 6), 7.51 (d, $J = 8.5$ Hz, 2 H, H 3', H 5'), 7.40 (d, $J = 8.5$ Hz, 2 H, H 2', H 6'), 5.32 (s, 2 H, CH₂O), 4.70 (s, 2 H, CH₂O), 3.97 (s, 3 H, NCH₃); MS (FAB⁺) m/z 472 (MH⁺, 1.5%); HRMS (FAB⁺) calc. for C₂₀H₁₈N₅O₉ (MH⁺) m/z 472.1105, found 472.1108.

4-(((1-Methyl-5-nitro-1H-imidazol-2-yl)methoxy)carbonyl)amino)benzyl doxorubicin carbamate (142). A solution of carbonate **141** (61 mg, 129 μ mol) in DMF (2 mL) was added dropwise to a stirred solution of doxorubicin (**13**) (45 mg, 86 μ mol) and Et₃N (18 μ L, 129 μ mol) in DMF (5 mL) at 20 °C and the solution stirred for 24 h. The solvent was evaporated and the residue purified by chromatography, eluting with 5% MeOH/DCM, to give **142** (50 mg, 66%) as a red solid, mp (DCM) 170-173 °C; ¹H NMR [(CD₃)₂SO] δ 14.00 (s, 1 H, 6-OH), 13.24 (s, 1 H, 11-OH), 9.91 (s, 1 H, OCONH), 8.07 (s, 1 H, H 4'''), 7.86-7.90 (m, 2 H, H 1, H 2), 7.60-7.63 (m, 1 H, H 3), 7.40 (d, $J = 8.4$ Hz, 2 H, H 3'', H 5''), 7.23 (d, $J = 8.4$ Hz, 2 H, H 2'', H 6''), 6.81 (d, $J = 8.0$ Hz, 1 H, OCONH), 5.43 (s, 1 H, H 7), 5.27 (s, 2 H, CH₂O), 5.21 (d, $J = 2.9$ Hz, 1 H, H 1'), 4.92-4.95 (m, 1 H, 9-OH), 4.87 (s, 2 H, CH₂O), 4.84 (dd, $J = 6.3, 5.9$ Hz, 1 H, 14-OH), 4.69 (d, $J = 5.7$ Hz, 1 H, 4-OH), 4.58 (d, $J = 6.0$ Hz, 2 H, H 14), 4.12-4.18 (m, 1 H, H 5'), 3.97 (s, 3 H, OCH₃), 3.95 (s, 3 H, NCH₃), 3.68-3.75 (m, 1 H, H 3'), 3.43-3.46 (m, 1 H, H 4'), 2.98 (d, $J = 18.3$ Hz, 1 H, H 10), 2.91 (d, $J = 18.3$ Hz, 1 H, H 10), 2.20 (br d, $J = 14.1$ Hz, 1 H, H 8), 2.11 (dd, $J = 14.1$ Hz, 1 H, H 8), 1.84 (dt, $J = 12.9, 3.7$ Hz, 1 H, H 2'), 1.47 (dd, $J = 12.9, 4.0$ Hz, 1 H, H 2'), 1.12 (d, J

= 6.5 Hz, 3 H, H 6'); ^{13}C NMR $[(\text{CD}_3)_2\text{SO}]$ δ 213.7 (C 13), 186.4 (C 5), 186.3 (C 12), 160.7 (C 4), 156.0 (C 6), 155.2 (C 11), 154.4 (OCONH), 152.4 (OCONH), 147.8 (C 5'''), 139.3 (C 2'''), 138.2 (C 4''), 136.1 (C 2), 135.4 (C 12a), 134.6 (C 6a), 134.0 (C 10a), 131.7 (C 4'''), 131.2 (C 1''), 128.6 (C 2'', C 6''), 119.9 (C 4a), 119.6 (C 1), 118.9 (C 3), 118.0 (C 3'', C 5''), 110.7 (C 5a), 110.5 (C 11a), 100.2 (C 1'), 74.9 (C 9), 69.8 (C 7), 67.9 (C 4'), 66.6 (C 5'), 64.8 (C 14), 63.6 (CH_2O), 57.6 (CH_2O), 56.5 (OCH_3), 47.0 (C 3'), 36.5 (C 8), 33.4 (NCH_3), 32.0 (C 10), 29.7 (C 2'), 16.9 (C 6'); MS (FAB $^+$) m/z 876 (MH^+ , 0.6%); HRMS (FAB $^+$) calc. for $\text{C}_{41}\text{H}_{42}\text{N}_5\text{O}_{17}$ (MH^+) m/z 876.2576, found 876.2573; Anal. ($\text{C}_{41}\text{H}_{41}\text{N}_5\text{O}_{17}\cdot\text{H}_2\text{O}$) C, H, N.

10

Example 5K. Preparation of [1-(2-hydroxyethyl)-5-nitro-1*H*-imidazol-2-yl]methyl 1-(chloromethyl)-3-[(5,6,7-trimethoxy-1*H*-indol-2-yl)carbonyl]-2,3-dihydro-1*H*-benzo[*e*]indol-5-ylcarbamate (148).

2-{5-Nitro-2-[(*E*)-2-phenylethenyl]-1*H*-imidazol-1-yl}ethanol (144). A solution of Na (2.0 g, 87.6 mmol) in dry MeOH (30 mL) was added in one portion to a stirred solution of metronidazole (143) (10.0 g, 58.4 mmol) and benzaldehyde (7.1 mL, 70 mmol) in DMSO (30 mL) at 20 °C. The mixture was stood at 20 °C for 24 h. Water (80 mL) was added and the resulting solid filtered. The solid was dissolved in EtOAc (100 mL) dried, and the solvent evaporated. The residue was purified by chromatography, eluted with 50% EtOAc/light petroleum, to give **144** (4.0 g, 26%) as a yellow powder, mp 155 °C (lit mp 156-157 °C [W. J. Ross, W. B. Jamieson, and M. C. McCowen, *J. Med. Chem.* **1972**, *15*, 1035-1039]); ^1H NMR δ 8.06 (s, 1 H, H 4'), 7.83 (d, J = 15.8 Hz, 1 H, CH=), 7.52-7.58 (m, 2 H, H 2'', H 6''), 7.33-7.38 (m, 3 H, H 3'', H 4'', H 5''), 7.05 (d, J = 15.8 Hz, 1 H, CH=), 4.64 (dd, J = 5.1, 5.0 Hz, 2 H, H 1), 4.07 (dd, J = 5.1, 5.0 Hz, 2 H, H 2), 2.42 (br s, 1 H, OH); ^{13}C NMR δ 150.9 (C 5'), 140.0 (CH=), 138.5 (C 2'), 135.3 (C 1''), 134.6 (C 4'), 129.7 (C 4''), 128.9 (C 3'', C 5''), 127.6 (C 2'', C 6''); 112.1 (CH=), 61.8 (C 1), 47.7 (C 2).

***tert*-Butyl(dimethyl)silyl 2-{5-nitro-2-[(*E*)-2-phenylethenyl]-1*H*-imidazol-1-yl}ethyl ether (145).** TBDMS triflate (2.7 mL, 11.75 mmol) was added dropwise to a stirred solution of alcohol **144** (2.77 g, 10.7 mmol) and pyridine (1.3 mL, 16.0 mmol) in DCM (100 mL) at -5 °C and the solution stirred at 20 °C for 16 h. The reaction was quenched with MeOH (5 mL) and poured in sat. aq. KHCO_3 (100 mL). The mixture was extracted with DCM (3 \times 50 mL), the combined organic fraction dried and the solvent evaporated.

- 99 -

The residue was purified by chromatography, eluting with 20% EtOAc/light petroleum, to give **145** (4.00 g, 100%) as a yellow solid, mp (EtOAc/light petroleum) 99-100.5 °C; ¹H NMR δ 8.13 (s, 1 H, H 4'), 7.87 (d, *J* = 15.8 Hz, 1 H, CH=), 7.57 (d, *J* = 6.8 Hz, 2 H, H 2'', H 6''), 7.34-7.41 (m, 3 H, H 3'', H 4'', H 5''), 7.06 (d, *J* = 15.8 Hz, 1 H, CH=), 4.62 (dd, *J* = 5.0, 4.8 Hz, 2 H, H 1), 4.00 (dd, *J* = 5.0, 4.8 Hz, 2 H, H 2), 0.77 (s, 9 H, SiC(CH₃)₃), 0.10 (s, 6 H, Si(CH₃)₂); ¹³C NMR δ 151.2 (C 5'), 139.3 (CH=), 138.4 (C 2'), 135.5 (C 1'), 134.8 (C 4'), 129.6 (C 4''), 128.9 (C 3'', C 5''), 127.5 (C 2'', C 6''), 112.9 (CH=), 62.3 (C 1), 47.8 (C 2), 25.7 (SiC(CH₃)₃), 18.1 (SiC(CH₃)₃), -5.8 (Si(CH₃)₂); Anal. (C₁₉H₂₇N₃O₃Si) C, H, N.

[1-(2-{{tert-Butyl(dimethyl)silyl}oxy}ethyl)-5-nitro-1*H*-imidazol-2-yl]methanol (146). Ozone was bubbled into a solution of imidazole **145** (1.3 g, 3.48 mmol) in DCM/MeOH (1:1, 120 mL) at -78 °C until a blue colour persisted. The solution was warmed to -40 °C with a N₂ purge to remove excess ozone. A solution of NaBH₄ (132 mg, 3.48 mmol) in EtOH (10 mL) was added dropwise over 15 min and the mixture stirred for 30 min. The mixture was treated with acetic acid (0.5 mL), stirred for 10 min and the solvent evaporated. The residue was partitioned between EtOAc (100 mL) and water (100 mL). The organic fraction was washed with water (50 mL), brine (25 mL), dried, and the solvent evaporated. The residue was purified by chromatography, eluting with 50% EtOAc/light petroleum, to give **146** (0.92 g, 88%) as a white solid, mp 104-105 °C; ¹H NMR δ 7.97 (s, 1 H, H 4), 4.79 (s, 2 H, CH₂O), 4.62 (t, *J* = 4.8 Hz, 2 H, CH₂O), 3.97 (t, *J* = 4.8 Hz, 2 H, CH₂N), 3.80 (br s, 1 H, OH), 0.81 (s, 9 H, SiC(CH₃)₃), 0.10 (s, 6 H, Si(CH₃)₂); ¹³C NMR δ 157.2 (C 2), 138.8 (C 5), 132.3 (C 4), 62.0 (CH₂O), 57.2 (CH₂O), 48.3 (CH₂N), 25.7 (SiC(CH₃)₃), 18.2 (SiC(CH₃)₃), -5.8 (Si(CH₃)₂); Anal. (C₁₂H₂₃N₃O₄Si) C, H, N.

[1-(2-{{tert-Butyl(dimethyl)silyl}oxy}ethyl)-5-nitro-1*H*-imidazol-2-yl]methyl 1-(chloromethyl)-3-[(5,6,7-trimethoxy-1*H*-indol-2-yl)carbonyl]-2,3-dihydro-1*H*-benzo[e]indol-5-ylcarbamate (147). A solution of triphosgene (25 mg, 84 μmol) in DCM (3 mL) was added dropwise to a stirred solution of amine **1** [G. J. Atwell, W. R. Wilson, W. A. Denny, *Bioorg. Med. Chem. Lett.*, **1997**, 7, 1483] (112 mg, 240 μmol) and Et₃N (67 μL, 481 μmol) in DCM (10 mL) and stirred at 20 °C for 2 h. A solution of alcohol **146** (78 mg, 264 μmol) in DCM (3 mL) was added, followed by nBu₂Sn(OAc)₂ (2 drops) and the solution stirred at 20 °C for 24 h. The solvent was evaporated and the residue purified by chromatography, eluting with 20% EtOAc/DCM, to give **147** (93 mg, 49%) as a colourless

- 100 -

oil, ^1H NMR δ 9.41 (s, 1 H, indole-NH), 8.88 (s, 1 H, OCONH), 8.06 (s, 1 H, H 4''), 7.87 (d, $J = 8.5$ Hz, 1 H, H 6), 7.79 (d, $J = 8.3$ Hz, 1 H, H 9), 7.57 (dd, $J = 8.3, 7.5$ Hz, 1 H, H 8), 7.46 (dd, $J = 8.5, 7.5$ Hz, 1 H, H 7), 7.17 (br s, 1 H, H 4), 7.00 (d, $J = 2.2$ Hz, 1 H, H 3'), 6.88 (s, 1 H, H 4'), 5.44 (d, $J = 13.5$ Hz, 1 H, CH_2O), 5.39 (d, $J = 13.5$ Hz, 1 H, CH_2O), 5 4.81 (dd, $J = 10.7, 1.5$ Hz, 1 H, H 2), 4.65-4.74 (m, 3 H, H 2, CH_2N), 4.16-4.22 (m, 1 H, H 1), 4.10 (s, 3 H, OCH_3), 3.95-3.99 (m, 4 H, CH_2Cl , OCH_3), 3.89-3.93 (m, 5 H, OCH_3 , CH_2N), 3.48 (t, $J = 10.9$ Hz, 1 H, CH_2Cl), 0.81 (s, 9 H, $\text{Si}(\text{CH}_3)_3$), -0.08 (s, 6 H, $\text{Si}(\text{CH}_3)_2$); MS (FAB $^+$) m/z 795 (MH^+ , 12%), 793 (25); HRMS (FAB $^+$) calc. for $\text{C}_{38}\text{H}_{46}^{35}\text{ClN}_6\text{O}_9\text{Si}$ (MH^+) m/z 793.2784, found 793.2762; calc. for $\text{C}_{38}\text{H}_{46}^{37}\text{ClN}_6\text{O}_9\text{Si}$ (MH^+) m/z 795.2755, found 795.2751.

[1-(2-hydroxyethyl)-5-nitro-1*H*-imidazol-2-yl]methyl 1-(chloromethyl)-3-[(5,6,7-trimethoxy-1*H*-indol-2-yl)carbonyl]-2,3-dihydro-1*H*-benzo[*e*]indol-5-ylcarbamate
 15 (148). 1M HCl (0.23 mL, 230 μmol) was added to a stirred solution of silyl ether 147 (91 mg, 115 μmol) in MeOH (5 mL) and the solution stirred at 20 °C for 4 h. The solvent was evaporated and the residue partitioned between EtOAc (40 mL) and water (40 mL). The organic fraction was washed with water (25 mL), brine (20 mL), dried, and the solvent evaporated. The residue was purified by chromatography, eluting with a gradient (0-
 20 10%MeOH/40%EtOAc/DCM), to give 148 as a white solid, mp (EtOAc/light petroleum) 148-150 (dec.); ^1H NMR δ [$(\text{CD}_3)_2\text{SO}$] 11.45 (s, 1 H, indole-NH), 9.92 (s, 1 H, OCONH), 8.45 (s, 1 H, H 4), 8.16 (s, 1 H, H 4''), 8.06 (d, $J = 8.5$ Hz, 1 H, H 6), 7.97 (d, $J = 8.3$ Hz, 1 H, H 9), 7.58 (ddd, $J = 8.3, 7.2, 0.8$ Hz, 1 H, H 8), 7.46 (ddd, $J = 8.5, 7.2, 0.8$ Hz, 1 H, H 7), 7.09 (d, $J = 2.0$ Hz, 1 H, H 3'), 6.97 (s, 1 H, H 4'), 5.37 (s, 2 H, CH_2O), 5.12 (t, $J = 5.4$
 25 Hz, 1 H, OH), 4.80 (dd, $J = 10.7, 9.4$ Hz, 1 H, H 2), 4.56-4.60 (m, 3 H, H 2, CH_2O), 4.32-4.38 (m, 1 H, H 1), 4.06 (dd, $J = 11.1, 3.2$ Hz, 1 H, CH_2Cl), 3.91-3.95 (m, 4 H, OCH_3 , CH_2Cl), 3.83 (s, 3 H, OCH_3), 3.81 (s, 3 H, OCH_3), 3.70-3.75 (m, 2 H, CH_2N); ^{13}C NMR δ [$(\text{CD}_3)_2\text{SO}$] 160.1 (CO), 154.0 (OCONH), 149.1 (C 5'), 148.7 (C 5''), 141.4 (C 3a), 139.9 (C 6'), 139.0 (C 7'), 138.9 (C 2''), 134.1 (C 5), 132.5 (C 4''), 130.7 (C 9a), 129.4 (C 2'),
 30 127.1 (C 8), 125.4 (C 7a'), 125.3 (C 5a), 124.3 (C 7), 123.8 (C 9), 123.3 (C 6), 123.1 (C 3a'), 122.2 (C 9b), 113.2 (C 4), 106.2 (C 3'), 98.0 (C 4'), 61.0 (OCH_3), 60.9 (OCH_3), 59.7 (CH_2O), 58.1 (CH_2O), 55.9 (OCH_3), 54.9 (C 2), 48.2 (CH_2N), 47.8 (CH_2Cl), 41.1 (C 1); MS (FAB $^+$) m/z 681 (MH^+ , 5%), 679 (MH^+ , 12%); HRMS (FAB $^+$) calc. for $\text{C}_{32}\text{H}_{32}^{35}\text{ClN}_6\text{O}_9$

(MH⁺) *m/z* 679.1919, found 679.1797; calc. for C₃₂H₃₂³⁷ClN₆O₉ (MH⁺) *m/z* 681.1890, found 681.1892; Anal. (C₃₂H₃₁ClN₆O₉) C, H, N.

Example 5L. Preparation of (1-methyl-5-nitro-1*H*-imidazol-4-yl)methyl 1-

- 5 (chloromethyl)-3-[(5,6,7-trimethoxy-1*H*-indol-1-yl)carbonyl]-2,3-dihydro-1*H*-benzo[*e*]indol-5-ylcarbamate (150).** A solution of triphosgene (21 mg, 70 μmol) in DCM (2 mL) was added dropwise to a stirred solution of amine 1 [G. J. Atwell, W. R. Wilson, W. A. Denny, *Bioorg. Med. Chem. Lett.*, 1997, 7, 1483] (93 mg, 200 μmol) and Et₃N (55 μL, 400 μmol) in DCM (10 mL) and stirred at 20 °C for 2 h. A solution of (1-methyl-5-nitro-
- 10 1*H*-imidazol-4-yl)methanol 149** [D. C. Baker, S.R. Putt, H. D. H. Showalter, *J. Heterocyclic Chem.*, 1983, 20, 629-634.] (37 mg, 240 μmol) in DCM (3 mL) was added, followed by nBu₂Sn(OAc)₂ (2 drops) and the solution stirred at 20 °C for 24 h. The solvent was evaporated and the residue purified by chromatography, eluting with 5% MeOH/EtOAc, to give 150 (40 mg, 56%) as a tan powder, mp (EtOAc) 219-220 °C; ¹H
- 15 NMR** [(CD₃)₂SO] δ 11.47 (s, 1 H, indole-NH), 9.80 (s, 1 H, OCONH), 8.54 (br s, 1 H, H 4), 8.08-8.11 (m, 2 H, H 6, H 2''), 7.97 (d, *J* = 8.4 Hz, 1 H, H 9), 7.57 (ddd, *J* = 8.4, 7.2, 0.7 Hz, 1 H, H 8), 7.45 (ddd, *J* = 8.5, 7.2, 0.7 Hz, 1 H, H 7), 7.09 (d, *J* = 2.0 Hz, 1 H, H 3'), 6.97 (s, 1 H, H 4'), 5.40 (s, 2 H, CH₂O), 4.79 (dd, *J* = 10.8, 1.4 Hz, 1 H, H 2), 4.52 (dd, *J* = 11.0, 1.9 Hz, 1 H, H 2), 4.31-4.36 (m, 1 H, H 1), 4.07 (dd, *J* = 11.1, 3.0 Hz, 1 H, CH₂Cl),
- 20 3.89-3.95 (m, 7 H, OCH₃, CH₂Cl, NCH₃), 3.83 (s, 3 H, OCH₃), 3.81 (s, 3 H, OCH₃); ¹³C NMR** [(CD₃)₂SO] δ 160.1 (CO), 154.3 (OCONH), 154.2 (C 5''), 149.1 (C 5'), 141.4 (C 2''), 141.2 (C 3a), 139.9 (C 6'), 139.0 (C 7'), 135.1 (C 4''), 134.4 (C 5), 130.8 (C 9a), 129.4 (C 2'), 127.0 (C 8), 125.5 (C 5a), 125.4 (C 7a'), 124.2 (C 7), 123.9 (C 9), 123.2 (C 6), 123.1 (C 3a'), 122.0 (C 9b), 113.1 (C 4), 106.2 (C 3'), 98.0 (C 4'), 61.0 (OCH₃), 60.9 (OCH₃), 59.6
- 25 (CH₂O), 55.9 (OCH₃), 54.8 (C 2), 47.5 (CH₂Cl), 41.1 (C 1), 35.1 (NCH₃); MS (FAB⁺) *m/z* 651 (MH⁺, 2%), 649 (MH⁺, 8); HRMS (FAB⁺) calc. for C₃₁H₃₀³⁵ClN₆O₈ (MH⁺) *m/z* 649.1814, found 649.1802; calc. for C₃₁H₃₀³⁷ClN₆O₈ (MH⁺) *m/z* 651.1784, found 651.1761; Anal. (C₃₁H₂₉ClN₆O₈) C, H, N.**

30 Example 6A. Preparation of (5-nitro-2-furyl)methyl 4-[bis(2-chloroethyl)amino]phenylcarbamate (154).

(5-Nitro-2-furyl)methyl 4-nitrophenyl carbonate (152). A solution of 4-nitrophenyl chloroformate (4.17 g, 20.7 mmol) in dry THF (50 mL) was added slowly to a stirred

- 102 -

solution of (5-nitrofuran-2-yl)methanol (**151**) [J. M. Berry, C. Y. Watson, W. J. D. Whish, and M. D. Threadgill. *J. Chem. Soc. Perkin Trans. I*, 1997, 1147] (2.69 g, 18.7 mmol) and pyridine (1.67 mL, 20.7 mmol) in THF (100 mL) at 20 °C under N₂. The mixture was stirred at 20 °C for 16 h, then partitioned between EtOAc (100 mL) and H₂O (100 mL). The organic layer was washed with saturated aqueous NaHCO₃ (50 mL), dried, and the solvent evaporated. The residue was purified by chromatography, eluting with a gradient (25-50%) of EtOAc/light petroleum to give **152** (4.79 g, 83%) as a white powder, mp (EtOAc/light petroleum) 93-94 °C; IR N 1775, 1526, 1352, 1215 cm⁻¹; ¹H NMR [(CD₃)₂SO] δ 8.34 (ddd, *J* = 9.2, 3.2, 2.1 Hz, 2 H, H 3, H 5), 7.72 (d, *J* = 3.8 Hz, 1 H, H 4'), 7.61 (ddd, *J* = 9.2, 3.2, 2.1 Hz, 2 H, H 2, H 6), 7.07 (d, *J* = 3.8 Hz, 1 H, H 3'), 5.43 (s, 2 H, CH₂O); ¹³C [(CD₃)₂SO] δ 155.0, 154.1, 151.8, 151.5, 145.2, 125.4(2), 122.5(2), 115.1, 113.3, 61.5; Anal. (C₁₂H₈N₂O₈) C, H, N.

(5-Nitro-2-furyl)methyl 4-[bis(2-hydroxyethyl)amino]phenylcarbamate (153). A solution of carbonate **152** (1.00 g, 3.24 mmol), *N*¹,*N*¹-bis(2-hydroxyethyl)-1,4-benzenediamine (**57**) (3.24 mmol), and pyridine (260 μL, 3.24 mmol) in THF (80 mL) was stirred at 20 °C for 16 h. The solvent was evaporated and the residue purified by chromatography, eluting with a gradient (0-10%) of MeOH/EtOAc, to give **153** (0.74 g, 63%) as an oil; ¹H NMR [(CD₃)₂SO] δ 9.44 (s, 1 H, OCONH), 7.68 (d, *J* = 3.7 Hz, 1 H, H 4'), 7.20 (br d, *J* = 9.1 Hz, 2 H, H 2, H 6), 6.93 (d, *J* = 3.7 Hz, 1 H, H 3'), 6.62 (d, *J* = 9.1 Hz, 2 H, H 3, H 5), 5.19 (s, 2 H, CH₂O), 4.71 (t, *J* = 5.4 Hz, 2 H, 2 × OH), 3.48-3.54 (m, 4 H, 2 × CH₂O), 3.33-3.38 (m, 4 H, 2 × CH₂N); ¹³C NMR [(CD₃)₂SO] δ 154.0, 152.7, 151.5, 144.2, 127.0, 120.4 (2), 113.8, 113.6, 111.4 (2), 58.1 (2), 57.1, 53.4 (2); MS (DEI) *m/z* 365 (M⁺, 15%), 334 (70), 222 (20), 196 (40), 191 (100); HRMS (DEI) calc. for C₁₆H₁₉N₃O₇ (M⁺) *m/z* 365.1223, found 365.1218.

(5-Nitro-2-furyl)methyl 4-[bis(2-chloroethyl)amino]phenylcarbamate (154). Methanesulphonyl chloride (460 μL, 6.0 mmol) was added dropwise to a stirred solution of diol **153** (0.73 g, 2.0 mmol) in pyridine (30 mL) at 5 °C and the solution stirred at 20 °C for 2 h. The solvent was evaporated and the residue partitioned between DCM (100 mL) and water (100 mL). The aqueous fraction was washed with DCM (2 × 50 mL), the combined organic extracts dried, and the solvent evaporated. The residue was dissolved in DMF (20 mL), LiCl (0.51 g, 12.0 mmol) added and the mixture stirred at 80 °C for 3 h. The solvent was

evaporated and the residue partitioned between EtOAc (150 mL) and water (150 mL). The aqueous fraction was extracted with EtOAc (2 × 80 mL), the combined extracts dried, and the solvent evaporated. The residue was purified by chromatography, eluting with 50% EtOAc/light petroleum, to give **154** (0.62 g, 77%) as an oil; ¹H NMR δ 9.57 (br s, 1 H, OCONH), 7.69 (d, *J* = 3.7 Hz, 1 H, H 4'), 7.28 (br d, *J* = 9.1 Hz, 2 H, H 2, H 6), 6.95 (d, *J* = 3.7 Hz, 1 H, H 3'), 6.70 (d, *J* = 9.1 Hz, 2 H, H 3, H 5), 5.21 (s, 2 H, CH₂O), 3.63-3.72 (m, 8 H, 2 × CH₂N, 2 × CH₂Cl); ¹³C NMR (CDCl₃) δ 153.9, 152.7, 151.5, 1142.3, 128.7, 120.3 (2), 113.8, 113.6, 112.3 (2), 57.2, 52.3 (2), 41.1 (2); MS (DEI) *m/z* 401 (M⁺, 50%), 403 (30), 405 (10) 354 (40), 352 (100); HRMS (DEI) calc. for C₁₆H₁₇³⁵Cl₂N₃O₅ (M⁺) *m/z* 401.0545, found 401.0546; calc. for C₁₆H₁₇³⁵Cl³⁷ClN₃O₅ (M⁺) *m/z* 403.0516, found 403.0521; calc. for C₁₆H₁₇³⁷Cl₂N₃O₅ (M⁺) *m/z* 405.0486, found 405.0498.

Example 6B. Preparation of (5-nitro-2-furyl)methyl 1-(chloromethyl)-3-[(5,6,7-trimethoxy-1*H*-indol-1-yl)carbonyl]-2,3-dihydro-1*H*-benzo[*e*]indol-5-ylcarbamate (155). A solution of triphosgene (14 mg, 48 μmol) in DCM (2 mL) was added dropwise to a stirred solution of amine **1** [G. J. Atwell, W. R. Wilson, W. A. Denny, *Bioorg. Med. Chem. Lett.*, **1997**, 7, 1483] (57 mg, 122 μmol) and Et₃N (38 μL, 275 μmol) in DCM (10 mL) and stirred at 20 °C for 2 h. A solution of (5-nitrofuran-2-yl)methanol (**151**) [J. M. Berry, C. Y. Watson, W. J. D. Whish, and M. D. Threadgill. *J. Chem. Soc. Perkin Trans. I*, **1997**, 1147] (24 mg, 165 μmol) in DCM (2 mL) was added, followed by nBu₂Sn(OAc)₂ (2 drops) and the solution stirred at 20 °C for 24 h. The solvent was evaporated and the residue purified by chromatography, eluting with 30% EtOAc/DCM, to give **155** (65 mg, 84%) as a white solid, mp (EtOAc/light petroleum) 185-187 °C; ¹H NMR δ 11.46 (s, 1 H, indole-NH), 9.92 (s, 1 H, OCONH), 8.54 (s, 1 H, H 4), 8.04 (d, *J* = 8.5 Hz, 1 H, H 6), 7.98 (d, *J* = 8.3 Hz, 1 H, H 9), 7.72 (d, *J* = 3.8 Hz, 1 H, H 4''), 7.58 (ddd, *J* = 8.3, 7.2, 0.7 Hz, 1 H, H 8), 7.45 (ddd, *J* = 8.5, 7.2, 0.7 Hz, 1 H, H 7), 7.09 (d, *J* = 2.1 Hz, 1 H, H 3'), 6.98-7.00 (m, 2 H, H 4', H 3''), 5.30 (s, 2 H, CH₂O), 4.80 (dd, *J* = 10.8, 9.5 Hz, 1 H, H 2), 4.53 (dd, *J* = 10.8, 1.9 Hz, 1 H, H 2), 4.32-4.37 (m, 1 H, H 1), 4.07 (dd, *J* = 11.1, 3.0 Hz, 1 H, CH₂Cl), 3.91-3.96 (m, 4 H, OCH₃, CH₂Cl), 3.83 (s, 3 H, OCH₃), 3.80 (s, 3 H, OCH₃); ¹³C NMR δ 160.1 (CO), 153.9 (OCONH), 153.8 (C 5''), 151.1 (C 2''), 149.2 (C 5'), 141.4 (C 3a), 139.8 (C 6'), 139.0 (C 7'), 134.0 (C 5), 130.7 (C 9a), 129.4 (C 2'), 127.1 (C 8), 125.4 (C 5a), 125.3 (C 7a'), 124.3 (C 7), 123.7 (C 9), 123.3 (C 6), 123.1 (C 3a), 122.2 (C 9b), 113.9 (C 3''), 113.6 (C 4''), 113.1 (C 4), 106.2 (C 3'), 98.0 (C 4'), 61.0 (OCH₃), 60.9 (OCH₃), 57.7

(CH₂O), 55.9 (OCH₃), 54.8 (C 2), 47.5 (CH₂Cl), 41.1 (C 1); MS (FAB⁺) *m/z* 635 (MH⁺, 6%), 637 (MH⁺, 3); HRMS (FAB⁺) calcd for C₃₁H₂₈³⁵ClN₄O₉ (MH⁺) *m/z* 635.1545, found 635.1552; calcd for C₃₁H₂₈³⁷ClN₄O₉ (MH⁺) *m/z* 637.1515, found 637.1514; Anal. (C₃₁H₂₇ClN₄O₉)

5

Example 7A. Preparation of (5-nitro-2-thienyl)methyl 4-[bis(2-chloroethyl)amino]phenylcarbamate (159).

4-Nitrophenyl (5-nitro-2-thienyl)methyl carbonate (157). 4-Nitrophenyl chloroformate (2.58 g, 12.8 mmol) in dry THF (20 mL) was added slowly to a stirred solution of (5-nitrothien-2-yl)methanol (156) [P. J. Narcombe, R. K. Norris. *Aust. J. Chem.* **1979**, *32*, 2647] (1.85 g, 11.6 mmol) and pyridine (1.03 mL, 12.8 mmol) in THF (50 mL) at 20 °C under N₂. The mixture was stirred at 20 °C for 16 h, then partitioned between EtOAc (100 mL) and H₂O (100 mL). The organic layer was washed with saturated aqueous NaHCO₃ (50 mL), and the solvent evaporated to give **157** (1.86g, 49%), mp (EtOAc/light petroleum) 121-122 °C; IR N 1763, 1522, 1345, 1231 cm⁻¹; ¹H NMR [(CD₃)₂SO] δ 8.33 (ddd, *J* = 9.2, 3.4, 2.2 Hz, 2 H, H 3', H 5'), 8.08 (d, *J* = 4.2 Hz, 1 H, H 4), 7.60 (ddd, *J* = 9.2, 3.4, 2.2 Hz, 2 H, H 2', H 6'), 7.4 (d, *J* = 4.2 Hz, 1 H, H 3), 5.56 (s, 2 H, CH₂O); ¹³C NMR [(CD₃)₂SO] δ 155.0, 151.6, 148.2, 145.2, 144.8, 129.5, 129.0, 125.4 (2), 122.6 (2), 64.4; Anal. (C₁₂H₈N₂O₇S) C, H, N.

20

(5-Nitro-2-thienyl)methyl 4-[bis(2-hydroxyethyl)amino]phenylcarbamate (158). A solution of **157** (0.75 g, 2.3 mmol), *N*¹,*N*¹-bis(2-hydroxyethyl)-1,4-benzenediamine (**57**) (2.5 mmol), and pyridine (206 μL, 2.5 mmol) in THF (50 mL) was stirred at 20 °C for 16 h. The solvent was evaporated and the residue purified by chromatography, eluting with EtOAc to give **158** (0.56 g, 64%), mp (EtOAc/light petroleum) 139-140.5 °C; IR N 3360, 3208, 1730, 1530, 1337, 1215 cm⁻¹; ¹H NMR [(CD₃)₂SO] δ 9.46 (s, 1 H, OCONH), 8.02 (d, *J* = 4.2 Hz, 1 H, H 4'), 7.29 (d, *J* = 4.2 Hz, 1 H, H 3'), 7.21 (br d, *J* = 9.1 Hz, 2 H, H 2, H 6), 6.62 (d, *J* = 9.1 Hz, 2 H, H 3, H 5), 5.33 (s, 2 H, CH₂O), 4.72 (t, *J* = 5.5 Hz, 2 H, 2 × OH), 3.49-3.56 (m, 4 H, 2 × CH₂O), 3.36 (t, *J* = 6.2 Hz, 4 H, 2 × CH₂N); ¹³C NMR [(CD₃)₂SO] δ 153.0, 150.8, 148.1, 144.3, 129.6, 127.5, 126.9, 120.5 (2), 111.4 (2), 60.1, 58.2 (2), 53.4 (2); Anal. (C₁₆H₁₉N₃O₆S) C, H, N, calc 11.0, found 10.5%.

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(5-Nitro-2-thienyl)methyl 4-[bis(2-chloroethyl)amino]phenylcarbamate (159).

- 105 -

Methane-sulphonyl chloride (260 μ L, 3.4 mmol) was added dropwise to a stirred solution of diol **158** (0.43 g, 1.1 mmol) in pyridine (10 mL) at 5 °C and the solution stirred at 20 °C for 2 h. The solvent was evaporated and the residue partitioned between DCM (50 mL) and water (50 mL). The aqueous fraction was washed with DCM (2 \times 50 mL), the combined organic extracts dried, and the solvent evaporated. The residue was dissolved in DMF (10 mL), LiCl (0.29 g, 6.8 mmol) added and the mixture stirred at 80 °C for 3 h. The solvent was evaporated and the residue partitioned between EtOAc (100 mL) and water (100 mL). The aqueous fraction was extracted with EtOAc (2 \times 50 mL), the combined extracts dried, and the solvent evaporated. The residue was purified by chromatography, eluting with 50% EtOAc/light petroleum, to give **159** (0.35 g, 69%) as pale green needles, mp (EtOAc/light petroleum) 99-100 °C; IR N 3353, 1723, 1547, 1530, 1339, 1219 cm^{-1} ; ^1H NMR [(CD₃)₂SO] δ 9.58 (br s, 1 H, OCONH), 8.04 (d, J = 4.2 Hz, 1 H, H 4'), 7.28-7.30 (m, 3 H, H 3', H 3, H 5), 6.71 (d, J = 9.1 Hz, 2 H, H 2, H 6), 5.34 (s, 2 H, CH₂O), 3.65-3.72 (m, 8 H, 2 \times CH₂N, 2 \times CH₂Cl); ^{13}C NMR [(CD₃)₂SO] δ 153.0, 150.8, 148.2, 142.4, 129.5, 128.6, 127.5, 120.4 (2), 112.3 (2), 60.1, 52.2 (2), 41.1 (2); Anal. (C₁₆H₁₉Cl₂N₅O₄) C, H, N, Cl.

Example 7B. Preparation of (5-nitro-2-thienyl)methyl 1-(chloromethyl)-3-[(5,6,7-trimethoxy-1*H*-indol-1-yl)carbonyl]-2,3-dihydro-1*H*-benzo[*e*]indol-5-ylcarbamate (160). A solution of triphosgene (15 mg, 51 μ mol) in DCM (2 mL) was added dropwise to a stirred solution of amine **1** [G. J. Atwell, W. R. Wilson, W. A. Denny, *Bioorg. Med. Chem. Lett.*, **1997**, 7, 1483] (61 mg, 131 μ mol) and Et₃N (41 μ L, 294 μ mol) in DCM (10 mL) and stirred at 20 °C for 2 h. A solution of (5-nitro-2-thienyl)methanol (**156**) [P. J. Narcombe, R. K. Norris. *Aust. J. Chem.* **1979**, 32, 2647] (28 mg, 176 μ mol) in DCM (2 mL) was added, followed by nBu₂Sn(OAc)₂ (2 drops) and the solution stirred at 20 °C for 24 h. The solvent was evaporated and the residue purified by chromatography, eluting with 10% EtOAc/DCM, to give **160** (76 mg, 89%) as a white solid, mp (EtOAc/light petroleum) 218-219 °C; ^1H NMR [(CD₃)₂SO] δ 11.48 (br s, 1 H, indole-NH), 9.34 (br s, 1 H, OCONH), 8.55 (br s, 1 H, H 4), 8.08 (d, J = 4.1 Hz, 1 H, H 4''), 8.04 (d, J = 8.5 Hz, 1 H, H 6), 7.99 (d, J = 8.3 Hz, 1 H, H 9), 7.58 (dd, J = 8.3, 7.4 Hz, 1 H, H 8), 7.47 (dd, J = 8.5, 7.4 Hz, 1 H, H 7), 7.33 (d, J = 4.1 Hz, 1 H, H 3''), 7.10 (d, J = 1.9 Hz, 1 H, H 3'), 6.97 (s, 1 H, H 4'), 5.43 (s, 2 H, CH₂O), 4.80 (dd, J = 11.0, 9.5 Hz, 1 H, H 2), 4.54 (dd, J = 11.0, 1.8 Hz, 1 H, H 2), 4.32-4.36 (m, 1 H, H 1), 4.07 (dd, J = 11.0, 3.0 Hz, 1 H, CH₂Cl), 3.92-3.96 (m, 4 H, CH₂Cl, OCH₃), 3.92 (m, 3 H, OCH₃), 3.81 (s, 3 H, OCH₃); ^{13}C NMR [(CD₃)₂SO] δ 160.3

- 106 -

- (CO), 154.4 (OCONH), 151.1 (C 5''), 149.3 (C 5'), 148.2 (C 2''), 141.6 (C 3a), 140.0 (C 6'), 139.1 (C 7'), 134.1 (C 5), 130.8 (C 9a), 129.7 (C 3''), 129.6 (C 2'), 127.8 (C 4''), 127.4 (C 8), 125.6 (C 5a), 125.5 (C 7a'), 124.6 (C 7), 123.8 (C 9), 123.3 (C 6), 123.2 (C 3a'), 122.5 (C 9b), 113.5 (C 4), 106.4 (C 3'), 98.1 (C 4'), 61.2 (OCH₃), 61.0 (OCH₃), 60.8 (CH₂O), 56.0 (OCH₃), 55.0 (C 2), 47.7 (CH₂Cl), 41.2 (C 1); MS (FAB⁺) *m/z* 653 (MH⁺, 4%), 651 (MH⁺, 8); HRMS (FAB⁺) calc. for C₃₁H₂₈³⁵ClN₄O₈S (MH⁺) *m/z* 651.1316, found 651.1311; calc. for C₃₁H₂₈³⁷ClN₄O₈S (MH⁺) *m/z* 653.1287, found 653.1307; Anal. (C₃₁H₂₇ClN₄O₈S) C, H, N.
- 10 **Example 8. Preparation of (1-methyl-5-nitro-1*H*-pyrazol-4-yl)methyl 1-(chloromethyl)-3-[(5,6,7-trimethoxy-1*H*-indol-1-yl)carbonyl]-2,3-dihydro-1*H*-benzo[*e*]indol-5-ylcarbamate (163).**
- (1-methyl-5-nitro-1*H*-pyrazol-4-yl)methanol (162). Borane dimethylsulfide (2 M solution in THF, 4.2 mL, 8.4 mmol) was added to a solution of 1-methyl-5-nitro-1*H*-pyrazole-4-carboxylic acid (161) [C.C. Cheng, *J. Heterocyclic Chem.* **1968**, *5*, 195-197] (1.11 g, 6.5 mmol) in THF (50 mL) under N₂, and the mixture stirred at reflux temperature for 80 min, then cooled. MeOH (5 mL), then water (5 mL), then 2 M HCl (5 mL) were added, the THF was evaporated, and the residue was diluted with water and extracted with EtOAc (3 × 50 mL). The combined organic extract was dried, the solvent evaporated, and the residue purified by chromatography, eluting with 50% EtOAc/petroleum ether, to give 162 (0.52 g, 51%) as a white solid, mp (benzene) 78-80 °C. ¹H NMR δ 7.58 (s, 1 H, H 3), 4.82 (d, *J* = 3.4 Hz, 2 H, CH₂O), 4.25 (s, 3 H, NCH₃), 2.39 (br s, 1 H, OH); Anal. (C₅H₇N₃O₃) C, H, N.
- 25 (1-Methyl-5-nitro-1*H*-pyrazol-4-yl)methyl 1-(chloromethyl)-3-[(5,6,7-trimethoxy-1*H*-indol-1-yl)carbonyl]-2,3-dihydro-1*H*-benzo[*e*]indol-5-ylcarbamate (163). A solution of triphosgene (14.3 mg, 48 μmol) in DCM (2 mL) was added dropwise to a stirred solution of amine 1 [G. J. Atwell, W. R. Wilson, W. A. Denny, *Bioorg. Med. Chem. Lett.*, **1997**, *7*, 1483] (57 mg, 122 μmol) and Et₃N (38 μL, 275 μmol) in DCM (10 mL) and stirred at 20 °C for 2 h. A solution of alcohol 162 (26 mg, 165 μmol) in DCM (2 mL) was added, followed by nBu₂Sn(OAc)₂ (2 drops) and the solution stirred at 20 °C for 24 h. The solvent was evaporated and the residue purified by chromatography, eluting with 20 % EtOAc/DCM, to give 163 (41 mg, 52%) as a white solid, mp (EtOAc/light petroleum) 201-

202 °C; ¹H NMR [(CD₃)₂SO] δ 11.47 (br s, 1 H, indole-NH), 9.80 (br s, 1 H, OCONH), 8.56 (br s, 1 H, H 4), 8.06 (d, *J* = 8.5 Hz, 1 H, H 6), 7.98 (d, *J* = 8.3 Hz, 1 H, H 9), 7.74 (br s, 1 H, H 3''), 7.58 (dd, *J* = 8.3, 7.4 Hz, 1 H, H 8), 7.47 (dd, *J* = 8.5, 7.4 Hz, 1 H, H 7), 7.45 (d, *J* = 1.6 Hz, 1 H, H 3'), 6.98 (s, 1 H, H 4'), 5.33 (s, 2 H, CH₂O), 4.80 (dd, *J* = 11.0, 9.4 Hz, 1 H, H 2), 4.53 (dd, *J* = 11.0, 1.8 Hz, 1 H, H 2), 4.32-4.38 (m, 1 H, H 1), 4.17 (s, 3 H, NCH₃), 4.07 (dd, *J* = 11.0, 3.1 Hz, 1 H, CH₂Cl), 3.91-3.96 (m, 4 H, OCH₃, CH₂Cl), 3.82 (s, 3 H, OCH₃), 3.80 (s, 3 H, OCH₃); ¹³C NMR [(CD₃)₂SO] δ 160.2 (CO), 154.3 (OCONH), 149.1 (C 5'), 142.5 (C 5''), 141.5 (C 3a), 139.9 (C 6'), 139.0 (C 7'), 137.5 (C 3''), 134.2 (C 5), 130.7 (C 9a), 129.4 (C 2'), 127.1 (C 8), 125.4 (C 5a, C 7a), 124.3 (C 7), 123.7 (C 9), 123.3 (C 6), 123.1 (C 3a'), 122.0 (C 9b), 117.4 (C 4''), 113.0 (C 4), 106.2 (C 3'), 98.0 (C 4'), 61.0 (OCH₃), 60.9 (OCH₃), 57.2 (CH₂O), 55.9 (OCH₃), 54.8 (C 2), 47.5 (CH₂Cl), 41.1 (C 1), 40.8 (NCH₃); MS (FAB⁺) *m/z* 650 (MH⁺, 2%), 648 (MH⁺, 5); HRMS (FAB⁺) calc. for C₃₁H₃₀³⁵ClN₆O₈ (MH⁺) *m/z* 649.1814, found 649.1803; calc. for C₃₁H₃₀³⁷ClN₆O₈ (MH⁺) *m/z* 651.1784, found 651.1796; Anal. (C₃₁H₂₉ClN₆O₈) C, H, N.

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Example 9A. Preparation of ethyl 4-((((1-(chloromethyl)-3-[(5,6,7-trimethoxy-1*H*-indol-1-yl)carbonyl]-2,3-dihydro-1*H*-benzo[*e*]indol-5-yl)amino)carbonyl)oxy)methyl)-1-methyl-5-nitro-1*H*-pyrrole-2-carboxylate (167).

Ethyl 4-formyl-1-methyl-5-nitro-1*H*-pyrrole-2-carboxylate (165). Dimethyl sulfate (0.31 mL, 3.2 mmol) was added to a mixture of ethyl 4-formyl-5-nitro-1*H*-pyrrole-2-carboxylate (164) [P. Fornari, M. Farnier, C. Fournier, *Bull. Soc. Chim. Fr.* 1972, 283-291] (0.57 g, 2.7 mmol) and K₂CO₃ (0.56 g, 4.0 mmol) in DMSO (4 mL) and the brown suspension was stirred at 20 °C for 1 h. The mixture was diluted with water (50 mL), acidified with HCl (2 N), and extracted with EtOAc (2 × 50 mL). The combined extract was dried, the solvent evaporated. The residue was chromatographed, eluting with 10%EtOAc/light petroleum, to give 165 (0.53 g, 86%) as a pale green solid, mp (benzene/light petroleum) 59-60.5 °C; ¹H NMR δ 10.32 (s, 1 H, CHO), 7.42 (s, 1 H, H 3), 4.37 (q, *J* = 7.1 Hz, 2 H, CH₂), 4.33 (s, 3 H, NCH₃), 1.39 (t, *J* = 7.1 Hz, 3 H, CH₃); Anal. (C₉H₁₀N₂O₅) C, H, N.

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Ethyl 4-(hydroxymethyl)-1-methyl-5-nitro-1*H*-pyrrole-2-carboxylate (166). NaBH₄ (0.33 g, 8.7 mmol) was added in portions to a solution of aldehyde 165 (3.96 g, 17.5 mmol) in EtOH (100 mL) and the mixture was stirred at 20 °C for 20 min. Water (5 mL) was

- slowly added, the EtOH was evaporated, and the residue was diluted with aq. NaCl and extracted with EtOAc (2 × 50 mL). The combined extract was washed with aq. NaCl (50 mL), dried, and the solvent evaporated. The residue was recrystallized to give **166** (1.40 g, 35%) as white needles, mp (benzene) 95.5-96.5 °C; ¹H NMR δ 7.01 (s, 1 H, H 3), 4.80 (br s, 2 H, CH₂O), 4.36 (q, *J* = 7.1 Hz, 2 H, CH₂), 4.31 (s, 3 H, NCH₃), 2.49 (br s, 1 H, OH), 1.38 (t, *J* = 7.1 Hz, 3 H, CH₃); Anal. (C₉H₁₂N₂O₅) C, H, N. The mother liquor was evaporated and purified by chromatography 10% EtOAc/light petroleum to give more **166** (1.46 g, 37%).
- 10 **Ethyl 4-(((1-(chloromethyl)-3-[(5,6,7-trimethoxy-1*H*-indol-1-yl)carbonyl]-2,3-dihydro-1*H*-benzo[*e*]indol-5-yl)amino)carbonyl)oxy)methyl)-1-methyl-5-nitro-1*H*-pyrrole-2-carboxylate (**167**). A solution of triphosgene (13.8 mg, 46 μmol) in DCM (2 mL) was added dropwise to a stirred solution of amine **1** [G. J. Atwell, W. R. Wilson, W. A. Denny, *Bioorg. Med. Chem. Lett.*, 1997, 7, 1483] (55 mg, 118 μmol) and Et₃N (37 μL, 265 μmol) in DCM (10 mL) and stirred at 20 °C for 2 h. A solution of ethyl 4-(hydroxymethyl)-1-methyl-5-nitro-1*H*-pyrrole-2-carboxylate (**166**) (36 mg, 159 μmol) in DCM (2 mL) was added, followed by nBu₂Sn(OAc)₂ (2 drops) and the solution stirred at 20 °C for 24 h. The solvent was evaporated and the residue purified by chromatography, eluting with 40% EtOAc/DCM, to give **167** (26 mg, 31%) as a white solid, mp (EtOAc/light petroleum) 248-250 °C; ¹H NMR [(CD₃)₂SO] δ 11.45 (s, 1 H, indole-NH), 9.86 (s, 1 H, OCONH), 8.56 (br s, 1 H, H 4''), 8.09 (d, *J* = 8.5 Hz, 1 H, H 6''), 7.99 (d, *J* = 8.3 Hz, 1 H, H 9''), 7.59 (dd, *J* = 8.3, 7.7 Hz, 1 H, H 8''), 7.48 (dd, *J* = 8.5, 7.7 Hz, 1 H, H 7''), 7.10 (d, *J* = 2.0 Hz, 1 H, H 3'''), 7.06 (br s, 1 H, H 3), 6.97 (s, 1 H, H 4'''), 5.38 (s, 2 H, CH₂O), 4.80 (dd, *J* = 11.0, 9.6 Hz, 1 H, H 2''), 4.53 (dd, *J* = 11.0, 2.0 Hz, 1 H, H 2''), 4.35-4.40 (m, 1 H, H 1''), 4.29 (q, *J* = 7.1 Hz, 2 H, H 1'), 4.19 (s, 3 H, NCH₃), 4.07 (dd, *J* = 11.0, 3.0 Hz, 1 H, CH₂Cl), 3.92-3.96 (m, 4 H, CH₂Cl, OCH₃), 3.83 (m, 3 H, OCH₃), 3.81 (s, 3 H, OCH₃), 1.31 (t, *J* = 7.1 Hz, 3 H, H 2'); ¹³C NMR [(CD₃)₂SO] δ 160.2 (CO), 159.4 (CO₂), 154.3 (OCONH), 149.1 (C 5'''), 141.5 (C 3a''), 139.9 (C 6'''), 139.0 (C 7'''), 137.1 (C 5), 134.2 (C 5''), 133.9 (C 2), 130.7 (C 9a''), 129.5 (C 2'''), 127.1 (C 8''), 125.6 (C 5a''), 125.5 (C 4), 125.4 (C 7a''), 124.3 (C 7''), 123.7 (C 9''), 123.3 (C 6''), 123.1 (C 3a'''), 122.1 (C 9b''), 114.6 (C 3), 113.0 (C 4''), 106.2 (C 3'''), 98.0 (C 4'''), 61.2 (C 1'), 61.0 (OCH₃), 60.9 (OCH₃), 59.6 (CH₂O), 55.9 (OCH₃), 54.9 (C 2''), 47.5 (CH₂Cl), 41.1 (C 1''), 35.2 (NCH₃), 14.0 (C 2'); MS (FAB⁺) *m/z* 722 (MH⁺, 0.3%), 720 (MH⁺, 0.6); HRMS (FAB⁺) calc. for**

$C_{35}H_{35}^{35}ClN_5O_{10}$ (MH^+) m/z 720.2073, found 720.2059; calc. for $C_{35}H_{35}^{37}ClN_5O_{10}$ (MH^+) m/z 722.2043, found 722.2031; Anal. ($C_{35}H_{34}ClN_5O_{10}$) C, H, N.

Example 9B. Preparation of (1-methyl-2-nitro-1H-pyrrol-3-yl)methyl 1-

- 5 **(chloromethyl)-3-[(5,6,7-trimethoxy-1H-indol-1-yl)carbonyl]-2,3-dihydro-1H-benzo[e]indol-5-ylcarbamate (169).**

- (1-methyl-2-nitro-1H-pyrrol-3-yl)methanol (168). A solution of NaOH (2.7 g, 68 mmol) in water (15 mL) was added to a solution of ester 166 (1.02 g, 4.47 mmol) in EtOH (30 mL), and the mixture was stirred at 20 °C for 1 h. The EtOH was evaporated, and the aqueous phase washed with EtOAc (20 mL) and then acidified (HCl). The aqueous mixture was extracted with EtOAc (3 × 50 mL), the combined organic extract was dried and the solvent evaporated to give crude 4-(hydroxymethyl)-1-methyl-5-nitropyrrole-2-carboxylic acid (0.84 g, 96%) as a red-brown solid.
- 15 The acid was suspended in quinoline (6 mL) with Cu powder (0.44 g) and the mixture was heated at 170-180 °C for 50 min. The cooled mixture was diluted with HCl (2N), extracted with EtOAc (3 × 50 mL), the combined extract was dried, and the solvent evaporated. The residue was purified by chromatography, eluting 40% EtOAc/light petroleum, to give 168 as a pink solid (0.45 g, 64%), mp (benzene) 79-80.5 °C; 1H NMR δ 6.78 (d, J = 2.8 Hz, 1 H, H 5), 6.27 (d, J = 2.5 Hz, 1 H, H 4), 4.80 (d, J = 6.8 Hz, 2 H, CH_2O), 4.00 (s, 3 H, NCH_3), 2.75 (t, J = 6.8 Hz, 1 H, OH); ^{13}C NMR δ 131.6 (C 3), 129.1 (C 4), 109.0 (C 5), 58.8 (CH_2O), 38.6 (NCH_3); Anal. ($C_6H_8N_2O_3$) C, H, N.
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- (1-Methyl-2-nitro-1H-pyrrol-3-yl)methyl 1-(chloromethyl)-3-[(5,6,7-trimethoxy-1H-indol-1-yl)carbonyl]-2,3-dihydro-1H-benzo[e]indol-5-ylcarbamate (169). A solution of triphosgene (13.3 mg, 45 μ mol) in DCM (2 mL) was added dropwise to a stirred solution of amine 1 [G. J. Atwell, W. R. Wilson, W. A. Denny. *Bioorg. Med. Chem. Lett.*, 1997, 7, 1483] (53 mg, 114 μ mol) and Et_3N (36 μ L, 256 μ mol) in DCM (10 mL) and stirred at 20 °C for 2 h. A solution of (1-methyl-2-nitro-1H-pyrrol-3-yl)methanol (168) (24 mg, 153 μ mol) in DCM (2 mL) was added, followed by $nBu_2Sn(OAc)_2$ (2 drops) and the solution stirred at 20 °C for 24 h. The solvent was evaporated and the residue purified by chromatography, eluting with a gradient (10-20%) of EtOAc/DCM, to give 169 (21 mg, 28%) as a white solid, mp (EtOAc) 218-220 °C; 1H NMR [$(CD_3)_2SO$] δ 11.46 (br s, 1 H,
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- 110 -

indole-NH), 9.80 (br s, 1 H, OCONH), 8.55 (br s, 1 H, H 4), 8.09 (d, $J = 8.5$ Hz, 1 H, H 6), 7.98 (d, $J = 8.3$ Hz, 1 H, H 9), 7.58 (dd, $J = 8.3, 7.3$ Hz, 1 H, H 8), 7.48 (dd, $J = 8.5, 7.3$ Hz, 1 H, H 7), 7.33 (d, $J = 2.6$ Hz, 1 H, H 4''), 7.09 (d, $J = 1.9$ Hz, 1 H, H 3'), 6.98 (s, 1 H, H 4'), 6.37 (br s, 1 H, H 3''), 5.37 (s, 2 H, CH₂O), 4.80 (dd, $J = 11.0, 9.3$ Hz, 1 H, H 2), 4.52 (dd, $J = 11.0, 1.9$ Hz, 1 H, H 2), 4.31-4.37 (m, 1 H, H 1), 4.07 (dd, $J = 11.1, 3.0$ Hz, 1 H, CH₂Cl), 3.96 (s, 3 H, NCH₃), 3.91-3.94 (m, 4 H, CH₂Cl, OCH₃), 3.82 (s, 3 H, OCH₃), 3.80 (s, 3 H, OCH₃); ¹³C NMR [(CD₃)₂SO] δ 160.2 (CO), 154.4 (OCONH), 149.1 (C 5'), 141.5 (C 3a), 139.9 (C 6'), 139.0 (C 7'), 134.3 (C 5), 133.2 (C 2''), 130.7 (C 9a), 129.4 (C 2'), 127.1 (C 8), 125.4 (C 5a), 125.3 (C 7a'), 124.3 (C 7), 123.8 (C 9), 123.3 (C 6), 123.1 (C 3a'), 122.0 (C 9b), 113.0 (C 4), 108.1 (C 5''), 106.1 (C 3'), 98.0 (C 4'), 61.0 (OCH₃), 60.9 (OCH₃), 60.2 (CH₂O), 55.9 (OCH₃), 54.8 (C 2), 47.5 (CH₂Cl), 41.1 (C 1), 37.9 (NCH₃); MS (FAB⁺) m/z 650 (MH⁺, 1.5%), 648 (MH⁺, 3.5); HRMS (FAB⁺) calc. for C₃₂H₃₁³⁵ClN₅O₈ (MH⁺) m/z 648.1861, found 648.1844; calc. for C₃₂H₃₁³⁷ClN₅O₈ (MH⁺) m/z 650.1832, found 650.1826; Anal. (C₃₂H₃₀ClN₅O₈) C, H, N.

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Example 9C. Preparation of ethyl 5-((((1-(chloromethyl)-3-[(5,6,7-trimethoxy-1H-indol-1-yl)carbonyl]-2,3-dihydro-1H-benzo[e]indol-5-yl)amino)carbonyl]oxy)methyl)-1-methyl-4-nitro-1H-pyrrole-2-carboxylate (173).

Ethyl 5-formyl-1-methyl-4-nitro-1H-pyrrole-2-carboxylate (171). Dimethyl sulfate (0.31 mL, 3.2 mmol) was added to a mixture of ethyl 5-formyl-4-nitro-1H-pyrrole-2-carboxylate (170) [P. Fornari, M. Farnier, C. Fournier, *Bull. Soc. Chim. Fr.* **1972**, 283-291] (0.57 g, 2.7 mmol) and K₂CO₃ (0.56 g, 4.0 mmol) in DMSO (4 mL) and the brown suspension was stirred at 20 °C for 1 h. The mixture was diluted with water (50 mL), acidified with HCl (2 N), and extracted with EtOAc (2 × 50 mL). The combined extract was dried, the solvent evaporated. The residue was chromatographed, eluting with 10%EtOAc/light petroleum, to give **171** as a pale green solid (89%), mp (benzene/light petroleum) 70.5-71.5 °C; ¹H NMR δ 10.55 (s, 1 H, CHO), 7.49 (s, 1 H, H 3), 4.38 (q, $J = 7.2$ Hz, 2 H, CH₂), 4.33 (s, 3 H, NCH₃), 1.40 (t, $J = 7.2$ Hz, 3 H, CH₃); Anal. (C₉H₁₀N₂O₅) C, H, N.

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Ethyl 5-(hydroxymethyl)-1-methyl-4-nitro-1H-pyrrole-2-carboxylate (172). NaBH₄ (0.33 g, 8.7 mmol) was added in portions to a solution of aldehyde **171** (3.96 g, 17.5 mmol) in EtOH (100 mL) and the mixture was stirred at 20 °C for 20 min. Water (5 mL) was

slowly added, the EtOH was evaporated, and the residue was diluted with brine and extracted with EtOAc (2 × 50 mL). The combined extract was washed with brine (50 mL), dried, and the solvent evaporated. The residue was recrystallized to give **172** as a cream solid (81%), mp (benzene) 119-120.5 °C; ¹H NMR δ 7.48 (s, 1 H, H 3), 4.97 (d, *J* = 6.8 Hz, 2 H, CH₂O), 4.32 (q, *J* = 7.1 Hz, 2 H, CH₂), 4.06 (s, 3 H, NCH₃), 2.72 (t, *J* = 7.1 Hz, 1 H, OH), 1.37 (t, *J* = 7.1 Hz, 3 H, CH₃); Anal. (C₉H₁₂N₂O₅) C, H, N.

Ethyl 5-({[1-(chloromethyl)-3-[(5,6,7-trimethoxy-1*H*-indol-1-yl)carbonyl]-2,3-dihydro-1*H*-benzo[*e*]indol-5-yl]amino)carbonyl]oxy)methyl)-1-methyl-4-nitro-1*H*-pyrrole-2-carboxylate (**173**). A solution of triphosgene (13.5 mg, 46 μmol) in DCM (2 mL) was added dropwise to a stirred solution of amine **1** [G. J. Atwell, W. R. Wilson, W. A. Denny, *Bioorg. Med. Chem. Lett.*, 1997, 7, 1483] (54 mg, 116 μmol) and Et₃N (36 μL, 260 μmol) in DCM (10 mL) and stirred at 20 °C for 2 h. A solution of ethyl 5-(hydroxymethyl)-1-methyl-4-nitro-1*H*-pyrrole-2-carboxylate (**172**) (36 mg, 156 μmol) in DCM (2 mL) was added, followed by nBu₂Sn(OAc)₂ (2 drops) and the solution stirred at 20 °C for 24 h. The solvent was evaporated and the residue purified by chromatography, eluting with 40% EtOAc/DCM, to give **173** (52 mg, 62%) as a white solid, mp (EtOAc/light petroleum) 227-229 °C; ¹H NMR [(CD₃)₂SO] δ 11.46 (s, 1 H, indole-NH), 9.83 (s, 1 H, OCONH), 8.56 (br s, 1 H, H 4''), 8.02 (d, *J* = 8.5 Hz, 1 H, H 6''), 7.97 (d, *J* = 8.3 Hz, 1 H, H 9''), 7.57 (dd, *J* = 8.3, 7.4 Hz, 1 H, H 8''), 7.46 (dd, *J* = 8.5, 7.4 Hz, 1 H, H 7''), 7.43 (s, 1 H, H 3), 7.10 (d, *J* = 2.0 Hz, 1 H, H 3'''), 6.98 (s, 1 H, H 4'''), 5.63 (s, 2 H, CH₂O), 4.80 (dd, *J* = 11.0, 9.4 Hz, 1 H, H 2''), 4.53 (dd, *J* = 11.0, 1.9 Hz, 1 H, H 2'''), 4.33-4.37 (m, 1 H, H 1''), 4.29 (q, *J* = 7.1 Hz, 2 H, H 1'), 4.00-4.08 (m, 4 H, CH₂Cl, NCH₃), 3.91-3.95 (m, 4 H, CH₂Cl, OCH₃), 3.83 (m, 3 H, OCH₃), 3.80 (s, 3 H, OCH₃), 1.31 (t, *J* = 7.1 Hz, 3 H, H 2'); ¹³C NMR [(CD₃)₂SO] δ 160.1 (CO), 159.3 (CO₂), 154.0 (OCONH), 149.1 (C 5'''), 141.4 (C 3a''), 139.9 (C 6'''), 139.0 (C 7'''), 134.1 (C 5''), 133.6 (C 4), 133.4 (C 2), 130.7 (C 9a''), 129.4 (C 2'''), 127.1 (C 8''), 125.4 (C 5a''), 125.3 (C 7a'''), 124.3 (C 7''), 123.7 (C 9''), 123.3 (C 6''), 123.1 (C 3a'''), 122.4 (C 5), 122.1 (C 9b''), 113.0 (C 4''), 111.2 (C 3), 106.2 (C 3'''), 98.0 (C 4'''), 61.0 (OCH₃), 60.9 (OCH₃), 60.8 (CH₂O), 55.9 (OCH₃), 54.8 (C 2''), 54.6 (C 1'), 47.5 (CH₂Cl), 41.1 (C 1''), 33.6 (NCH₃) 13.9 (C 2'); MS (FAB⁺) *m/z* 722 (MH⁺, 2.5%), 720 (MH⁺, 6); HRMS (FAB⁺) calc. for C₃₅H₃₅³⁵ClN₅O₁₀ (MH⁺) *m/z* 722.2073, found 720.2045; calc. for C₃₅H₃₅³⁷ClN₅O₁₀ (MH⁺) *m/z* 722.2043,

- 112 -

found 722.2039; Anal. (C₃₅H₃₄ClN₅O₁₀) C, H, N.

Example 9D. Preparation of (1-methyl-3-nitro-1H-pyrrol-2-yl)methyl 1-(chloromethyl)-3-[(5,6,7-trimethoxy-1H-indol-1-yl)carbonyl]-2,3-dihydro-1H-benzo[e]indol-5-ylcarbamate (175).

2-(Hydroxymethyl)-1-methyl-3-nitropyrrole (174). A solution of NaOH (1 M, 16.7 mL, 16.7 mmol) was added to a solution of ester 172 (0.76 g, 3.34 mmol) in EtOH (50 mL), and the mixture was stirred at 20 °C for 1 h. The EtOH was evaporated, water (50 mL) added and the aqueous phase washed with diethyl ether (50 mL). The pH of the aqueous phase was adjusted to 2 with 1 M HCl. The aqueous mixture was extracted with EtOAc (3 × 50 mL), the combined organic extract was dried and the solvent evaporated to give crude 4-(hydroxymethyl)-1-methyl-5-nitropyrrole-2-carboxylic acid (0.65 g, 97%) as a red-brown solid.

The acid was suspended in quinoline (10 mL) with Cu powder (0.50 g) and the mixture was heated at 180-190 °C for 50 min under N₂. The cooled mixture was diluted with EtOAc (50 mL) and 1 M HCl (50 mL), extracted with EtOAc (3 × 50 mL), the combined extract was dried, and the solvent evaporated. The residue was purified by chromatography, eluting 50% EtOAc/light petroleum, to give 174 as a pale yellow solid (0.29 g, 57%), mp 63-64 °C (benzene); ¹H NMR δ 6.73 (d, *J* = 3.4 Hz, 1 H, H 5), 6.50 (d, *J* = 3.4 Hz, 1 H, H 4), 4.89 (d, *J* = 7.2 Hz, 2 H, CH₂O), 3.73 (s, 3 H, NCH₃), 2.83 (t, *J* = 7.2 Hz, 1 H, OH); ¹³C NMR δ 134.9 (C 3), 133.5 (C 2), 122.0 (C 4), 105.7 (C 5), 53.8 (CH₂O), 34.8 (NCH₃); Anal. (C₆H₈N₂O₃) C, H, N.

(1-Methyl-3-nitro-1H-pyrrol-2-yl)methyl 1-(chloromethyl)-3-[(5,6,7-trimethoxy-1H-indol-1-yl)carbonyl]-2,3-dihydro-1H-benzo[e]indol-5-ylcarbamate (175). A solution of triphosgene (15 mg, 50 μmol) in DCM (2 mL) was added dropwise to a stirred solution of amine 1 [G. J. Atwell, W. R. Wilson, W. A. Denny, *Bioorg. Med. Chem. Lett.*, 1997, 7, 1483] (59 mg, 127 μmol) and Et₃N (40 μL, 284 μmol) in DCM (10 mL) and stirred at 20 °C for 2 h. A solution of alcohol 174 (27 mg, 171 μmol) in DCM (2 mL) was added, followed by nBu₂Sn(OAc)₂ (2 drops) and the solution stirred at 20 °C for 24 h. The solvent was evaporated and the residue purified by chromatography, eluting with a gradient (20-30%) of EtOAc/DCM, to give 175 (11 mg, 13%) as a white solid, mp (EtOAc) 218-220 °C; ¹H NMR δ 9.42 (s, 1 H, indole-NH), 8.94 (s, 1 H, OCONH), 7.87 (d, *J* = 8.4 Hz, 1 H, H 6),

7.79 (d, $J = 8.2$ Hz, 1 H, H 9), 7.57 (ddd, $J = 8.2, 7.4, 0.9$ Hz, 1 H, H 8), 7.47 (ddd, $J = 8.4, 7.4, 0.9$ Hz, 1 H, H 7), 7.08 (br s, 1 H, H 4), 7.01 (d, $J = 2.2$ Hz, 1 H, H 3'), 6.89 (s, 1 H, H 4'), 6.80 (d, $J = 3.3$ Hz, 1 H, H 5''), 6.57 (d, $J = 3.3$ Hz, 1 H, H 4''), 5.65 (s, 2 H, CH₂O), 4.81 (dd, $J = 10.7, 91.7$ Hz, 1 H, H 2), 4.67 (dd, $J = 10.7, 8.6$ Hz, 1 H, H 2), 4.15-4.20 (m, 1
 5 H, H 1), 4.10 (s, 3 H, OCH₃), 3.95-3.99 (m, 4 H, OCH₃, CH₂Cl), 3.92 (s, 3 H, OCH₃), 3.80 (br s, 3 H, NCH₃), 3.48 (dd, $J = 11.0, 10.7$ Hz, 1 H, CH₂Cl); ¹³C NMR δ 160.4 (CO), 153.9 (OCONH), 150.2 (C 5'), 141.6 (C 3a), 140.6 (C 6'), 138.9 (C 7'), 133.7 (C 5), 130.9 (C 3''), 129.7 (C 9a), 129.6 (C 2'), 128.8 (C 2''), 127.5 (C 8), 125.6 (C 7a'), 125.1 (C 7, C 5a), 123.6 (C 3a'), 123.1 (C 9), 122.6 (C 6), 122.2 (C 4''), 121.6 (C 9b), 113.0 (C 4), 106.5 (C
 10 3'), 106.1 (C 5''), 97.7 (C 4'), 61.5 (OCH₃), 61.1 (OCH₃), 56.3 (OCH₃), 55.3 (CH₂O), 54.9 (C 2), 45.8 (CH₂Cl), 43.4 (C 1), 35.3 (NCH₃); MS (FAB⁺) m/z 650 (MH⁺, 0.6%), 648 (MH⁺, 1.5); HRMS (FAB⁺) calc. for C₃₂H₃₁³⁵ClN₅O₈ (MH⁺) m/z 648.1861, found 648.1850; calc. for C₃₂H₃₁³⁷ClN₅O₈ (MH⁺) m/z 650.1832, found 650.1841; Anal. (C₃₂H₃₀ClN₅O₈) C, H, N.

15

Example 9E. Preparation of (1-methyl-5-nitro-1H-pyrrol-2-yl)methyl 1-(chloromethyl)-3-[(5,6,7-trimethoxy-1H-indol-1-yl)carbonyl]-2,3-dihydro-1H-benzo[e]indol-5-ylcarbamate (178).

(1-Methyl-5-nitro-1H-pyrrol-2-yl)methanol (177). NaBH₄ (0.19 g, 5.03 mmol) was
 20 added to a stirred solution of 1-methyl-5-nitro-1H-pyrrole-2-carbaldehyde (176) [P. Fournari, *Bull. Soc. Chim. Fr.* **1963**, 488-491] (0.78 g, 5.07 mmol) in MeOH (40 mL) at room temperature under N₂. After addition was complete, the reaction mixture was stirred for a further 20 min, then water (40 mL) was added and the mixture was saturated with solid K₂CO₃. The mixture was extracted with EtOAc (3 × 50 mL), the combined organic
 25 fraction dried, and the solvent evaporated to give 177 (0.77 g, 97%) as a white solid, mp (EtOAc/light petroleum) 76-77 °C; ¹H NMR δ 7.16 (d, $J = 4.3$ Hz, 1 H, H 4), 6.17 (d, $J = 4.3$ Hz, 1 H, H 3), 4.68 (s, 2 H, CH₂), 4.02 (s, 3 H, CH₃); Anal. (C₆H₈N₂O₃) C, H, N.

(1-Methyl-5-nitro-1H-pyrrol-2-yl)methyl 1-(chloromethyl)-3-[(5,6,7-trimethoxy-1H-indol-1-yl)carbonyl]-2,3-dihydro-1H-benzo[e]indol-5-ylcarbamate (178). A solution of triphosgene (16 mg, 52 μ mol) in DCM (2 mL) was added dropwise to a stirred solution of amine 1 [G. J. Atwell, W. R. Wilson, W. A. Denny, *Bioorg. Med. Chem. Lett.*, **1997**, 7, 1483] (62 mg, 133 μ mol) and Et₃N (42 μ L, 299 μ mol) in DCM (10 mL) and stirred at 20

30

°C for 2 h. A solution of alcohol 177 (28 mg, 179 μ mol) in DCM (2 mL) was added, followed by $n\text{Bu}_2\text{Sn}(\text{OAc})_2$ (2 drops) and the solution stirred at 20 °C for 24 h. The solvent was evaporated and the residue purified by chromatography, eluting with 20%

EtOAc/DCM, to give 178 (54 mg, 63%) as a white solid, mp (EtOAc) 212-214 °C; ^1H

5 NMR $[(\text{CD}_3)_2\text{SO}]$ δ 11.45 (br s, 1 H, indole-NH), 9.82 (br s, 1 H, OCONH), 8.55 (br s, 1 H, H 4), 8.04 (d, $J = 8.5$ Hz, 1 H, H 6), 7.89 (d, $J = 8.3$ Hz, 1 H, H 9), 7.57 (dd, $J = 8.3, 7.2$ Hz, 1 H, H 8), 7.45 (dd, $J = 8.5, 7.2$ Hz, 1 H, H 7), 7.24 (d, $J = 4.4$ Hz, 1 H, H 4''), 7.09 (d, $J = 2.0$ Hz, 1 H, H 3'), 6.98 (s, 1 H, H 4'), 6.45 (d, $J = 4.4$ Hz, 1 H, H 3''), 5.30 (s, 2 H, CH_2O), 4.80 (dd, $J = 11.0, 9.4$ Hz, 1 H, H 2), 4.53 (dd, $J = 11.0, 1.8$ Hz, 1 H, H 2), 4.32-
10 4.37 (m, 1 H, H 1), 4.07 (dd, $J = 11.1, 3.1$ Hz, 1 H, CH_2Cl), 3.91-3.96 (m, 7 H, CH_2Cl , NCH_3 , OCH_3), 3.83 (s, 3 H, OCH_3), 3.81 (s, 3 H, OCH_3); ^{13}C NMR $[(\text{CD}_3)_2\text{SO}]$ δ 160.2 (CO), 154.0 (OCONH), 149.1 (C 5'), 142.1 (C 5''), 141.4 (C 3a), 139.9 (C 6'), 138.2 (C 7'), 136.1 (C 2''), 134.2 (C 5), 130.7 (C 9a), 129.4 (C 2'), 127.1 (C 8), 125.3 (C 5a), 125.2 (C 7a'), 124.3 (C 7), 123.7 (C 9), 123.3 (C 6), 123.1 (C 3a'), 122.1 (C 9b), 113.1 (C 3''),
15 113.0 (C 4), 110.6 (C 4''), 106.2 (C 3'), 98.0 (C 4'), 61.0 (OCH_3), 60.9 (OCH_3), 57.6 (CH_2O), 55.9 (OCH_3), 54.8 (C 2), 47.5 (CH_2Cl), 41.1 (C 1), 33.9 (NCH_3); MS (FAB $^+$) m/z 650 (MH^+ , 1%), 648 (MH^+ , 2); HRMS (FAB $^+$) calc. for $\text{C}_{32}\text{H}_{31}^{35}\text{ClN}_5\text{O}_8$ (MH^+) m/z 648.1861, found 648.1852; calc. for $\text{C}_{32}\text{H}_{31}^{37}\text{ClN}_5\text{O}_8$ (MH^+) m/z 650.1832, found 650.1836; Anal. ($\text{C}_{32}\text{H}_{30}\text{ClN}_5\text{O}_8$) C, H, N.

20

Elemental analysis data

	No	Formula	Calculated (%)	Found (%)
	1	$\text{C}_{21}\text{H}_{15}\text{N}_2\text{O}_5\text{S}$	C, 62.2; H, 3.7; N, 10.4	C, 62.3; H, 3.6; N, 10.7
25	2A	$\text{C}_{19}\text{H}_{21}\text{Cl}_2\text{N}_2\text{O}_5$	C, 51.6; H, 4.8; N, 9.5	C, 51.8; H, 4.9; N, 9.5
	2B	$\text{C}_{10}\text{H}_{29}\text{ClN}_4\text{O}_9$	C, 57.65; H, 4.7; N, 9.0	C, 57.45; H, 4.7; N, 8.7
	2C	$\text{C}_{14}\text{H}_{31}\text{ClN}_4\text{O}_9\text{H}_2\text{O}$	C, 59.1; H, 4.5; 8.1	59.1; H, 4.2; N, 7.9
	2D	$\text{C}_{22}\text{H}_{17}\text{N}_2\text{O}_5\text{S}$	C, 60.7; H, 3.9; N, 9.7	C, 60.6; H, 4.0; N, 9.8
	2E	$\text{C}_{36}\text{H}_{36}\text{N}_2\text{O}_{16}$	C, 57.4; H, 4.8; N, 3.7	C, 57.2; H, 5.1; N, 3.9
30	2F	$\text{C}_{44}\text{H}_{43}\text{N}_2\text{O}_{18}\text{H}_2\text{O}$	C, 57.45; H, 4.9	C, 57.4; H, 5.1
	2G	$\text{C}_{27}\text{H}_{17}\text{N}_2\text{O}_6$	C, 62.7; H, 5.3; N, 13.5	C, 62.1; H, 5.5; N, 13.4
	2H	$\text{C}_{45}\text{H}_{42}\text{N}_6\text{O}_7 \cdot \frac{1}{2}\text{H}_2\text{O}$	C, 68.6; H, 5.5; N, 10.7	C, 68.4; H, 5.6; N, 10.65
	2I	$\text{C}_{18}\text{H}_{18}\text{N}_6\text{O}_{14}$ (M^+)	802.2446	802.2446
	2J	$\text{C}_{20}\text{H}_{14}\text{N}_6\text{O}_{10}$ (MH^+)	624.2306	624.2297
35	3A	$\text{C}_{19}\text{H}_{21}^{35}\text{Cl}_2\text{N}_4\text{O}_4$ (M^+)	425.0909	425.0901
	3B	$\text{C}_{14}\text{H}_{31}\text{ClN}_4\text{O}_8$	C, 62.0; H, 4.7; N, 8.5	C, 61.9; H, 4.8; N, 8.3
	3C	$\text{C}_{20}\text{H}_{21}^{35}\text{Cl}_2\text{N}_4\text{O}_4$ (M^+)	455.1015	455.1017
	4A	$\text{C}_{15}\text{H}_{33}\text{ClN}_4\text{O}_{10}$	C, 59.6; H, 4.7; N, 7.95	C, 59.4; H, 4.9; N, 7.65
	4B	$\text{C}_{16}\text{H}_{35}\text{ClN}_4\text{O}_{10}$	C, 60.1; H, 4.9; N, 7.8	C, 60.3; H, 5.1; N, 7.5
40	4C	$\text{C}_{21}\text{H}_{25}\text{Cl}_2\text{N}_4\text{O}_6$	C, 51.9; H, 5.2; N, 8.6; Cl, 14.6	C, 51.95; H, 5.2; N, 8.6; Cl, 14.5
	4D	$\text{C}_{12}\text{H}_{33}\text{ClN}_4\text{O}_{10} \cdot \frac{1}{2}\text{H}_2\text{O}$	C, 56.7; H, 5.05; N, 8.3	C, 56.9; H, 4.8; N, 8.1
	4E	$\text{C}_{16}\text{H}_{35}\text{ClN}_4\text{O}_{10}$	C, 60.1; H, 4.9; N, 7.8	C, 60.2; H, 5.2; N, 7.6

5	4F	87	C ₃₈ H ₄₀ N ₂ O ₁₇ ·½H ₂	C, 56.6; H, 5.1; N, 3.5	C, 56.7; H, 5.1; N, 3.5
	4G	91	C ₄₇ H ₄₆ N ₂ O ₈ ·2HCl·2½H ₂	C, 60.0; H, 5.7; N, 8.9	C, 59.8; H, 5.95; N, 9.0
	4H	93	C ₃₆ H ₁₇ ³⁷ ClN ₄ O ₁₁ P (MH ⁺)	799.1783	799.1757
	4I	99	C ₃₆ H ₃₅ ClN ₄ O ₁₁ ·CH ₃ OH	C, 57.9; H, 5.1; N, 7.3	C, 57.8; H, 4.8; N, 7.2
	4J	102	C ₃₈ H ₄₁ ClN ₂ O ₉ ·2HCl	C, 55.7; H, 5.2; N, 8.55	C, 56.1; H, 5.5; N, 8.6
10	4K	105	C ₄₀ H ₄₂ ClN ₂ O ₁₀ ·½H ₂ O	C, 60.3; H, 5.4; N, 8.8	60.3; H, 5.5; N, 8.5
	4L	107	C ₅₁ H ₃₄ N ₂ O ₈ (M ⁺)	892.4034	892.4055
	4M	113	C ₄₈ H ₄₆ N ₆ O ₉ ·2HCl·2H ₂ O	C, 60.1; H, 5.45; N, 8.8	C, 59.8; H, 5.6; N, 8.8
	5A	117	C ₁₀ H ₁₄ Cl ₂ N ₄ O ₄	C, 36.9; H, 4.3; N, 17.2; Cl, 21.8	C, 37.4; H, 4.1; N, 17.2; Cl, 21.8
	5B	123	C ₁₆ H ₁₉ Cl ₂ N ₄ O ₄	C, 46.2; H, 4.6; N, 16.8; Cl, 17.0	C, 46.3; H, 4.8; N, 17.1; Cl, 17.1
15	5C	124	C ₃₁ H ₂₉ ClN ₆ O ₈ ·½H ₂ O	C, 56.6; H, 4.6; N, 12.8	C, 56.6; H, 4.4; N, 12.5
	5D	125	C ₃₃ H ₃₄ N ₄ O ₁₅ ·½H ₂ O	C, 53.9; H, 4.8; N, 7.6	C, 53.7; H, 4.8; N, 7.3
	5E	129	C ₄₁ H ₄₁ N ₂ O ₁₇ ·H ₂ O	C, 55.1; H, 4.85; N, 7.8	C, 54.7; H, 4.9; N, 7.5
	5F	132	C ₃₁ H ₂₉ ClN ₆ O ₈	C, 57.4; H, 4.5; N, 12.95	C, 57.7; H, 4.5; N, 12.9
	5G	135	C ₁₀ H ₁₄ Cl ₂ N ₄ O ₄ (M ⁺)	324.1392	324.1381
20	5H	137	C ₁₆ H ₁₉ Cl ₂ N ₄ O ₄	C, 46.2; H, 4.6; N, 16.8	C, 46.1; H, 4.6; N, 16.7
	5I	138	C ₃₁ H ₂₉ ClN ₆ O ₈	C, 57.4; H, 4.5; N, 12.95	C, 57.5; H, 4.6; N, 12.9
	5J	142	C ₄₁ H ₄₁ N ₂ O ₁₇ ·H ₂ O	C, 55.1; H, 4.85; N, 7.8	C, 55.2; H, 4.9; N, 7.9
	5K	148	C ₃₂ H ₃₁ ClN ₆ O ₈	C, 56.6; H, 4.6; N, 12.4	C, 56.5; H, 4.9; N, 12.1
	5L	150	C ₃₁ H ₂₉ ClN ₆ O ₈	C, 57.4; H, 4.5; N, 12.95	C, 57.2; H, 4.5; N, 12.9
25	6A	154	C ₁₆ H ₁₇ ³⁵ Cl ₂ N ₃ O ₅ (M ⁺)	401.0545	401.0546
	6B	155	C ₃₁ H ₂₇ ClN ₆ O ₈	C, 58.6; H, 4.3; N, 8.8	C, 58.5; H, 4.3; N, 8.9
	7A	159	C ₁₆ H ₁₉ Cl ₂ N ₄ O ₄	C, 45.9; H, 4.1; N, 10.1; Cl, 17.0	C, 46.2; H, 4.0; N, 9.9; Cl, 17.2
	7B	160	C ₃₁ H ₂₇ ClN ₆ O ₈ S	C, 57.2; H, 4.2; N, 8.6	C, 57.4; H, 4.0; N, 8.4
	8	163	C ₃₁ H ₂₉ ClN ₆ O ₈	C, 57.4; H, 4.5; N, 12.95	C, 57.5; H, 4.6; N, 13.1
30	9A	167	C ₃₅ H ₃₄ ClN ₂ O ₁₀	C, 58.4; H, 4.8; N, 9.7	C, 58.2; H, 4.9; N, 9.7
	9B	169	C ₃₂ H ₃₀ ClN ₂ O ₈	C, 59.3; H, 4.7; N, 10.8	C, 59.4; H, 4.8; N, 10.8
	9C	173	C ₃₅ H ₃₄ ClN ₂ O ₁₀	C, 58.4; H, 4.8; N, 9.7	C, 58.6; H, 4.9; N, 9.8
	9D	175	C ₃₂ H ₃₀ ClN ₂ O ₈	C, 59.3; H, 4.7; N, 10.8	C, 59.1; H, 4.7; N, 10.9
	9E	178	C ₃₂ H ₃₀ ClN ₂ O ₈	C, 59.3; H, 4.7; N, 10.8	C, 59.4; H, 4.8; N, 10.7

Example 10

Biological activity

Selected compounds were evaluated for cytotoxicity (measured as IC₅₀ values in μM following and 18 h drug exposure) in pairs of mammalian cell lines, and the results are given in Table 2. The human ovarian carcinoma line (SKOV3) is wild-type, while the SC3.2 line is the NR+ transfectant. The human colon carcinoma line NR- line (WIDR) is wild-type, while the WC14.10 line is the NR+ transfectant. The murine mammary carcinoma (EMT6-V) is wild-type, while the EN2A is the NR+ transfectant. Ratios (NR-/NR+) provide a major measure of efficacy of action.

Table 3. Biological activity for selected compounds.

Example	No	SKOV3	SKOV/ SC3.2	WiDr	WiDr/ WC14.10	EMT6-V	EMT6/ EN2A
		NR- IC ₅₀ (μmol)	IC ₅₀ ratios	NR- IC ₅₀ (μmol)	Ratio	NR- IC ₅₀ (μmol)	Ratio
2C	35	0.059	16.6	0.14	47	-	-
2E	38	2.45	5.9	2.78	3.1	-	-
2F	42	>10	-	>10	>16	3.33	>54
2G	46	1.81	3.9	1.21	3.3	0.76	49
2H	51	5.3	8.1	2.8	3.6	0.62	2.44

- 116 -

	3B	60	0.157	5.6	0.15	6.6	-	-
	4A	70	0.224	84	0.30	147	0.106	104
	4B	73	0.105	67	0.109	45	0.033	78
	4E	84	0.145	58	0.19	91	0.47	91
5	4G	91	9.4	>36	6.6	22	1.6	15.6
	4I	99	0.114	40	0.13	25	0.44	13.4
	4K	105	0.088	6.4	0.097	9	0.024	6.1
	5B	123	2.3	28	1.7	6.7	-	-
	5C	124	0.075	21	0.075	40	-	-
10	5D	125	3.74	14.5	3.59	10.3	0.46	6.4
	5E	129	>1.5	>12	>1.5	>5.6	0.24	4.95
	5I	138	0.15	50	0.23	99	0.078	71
	5J	142	>3	>9.0	2.72	1.64	0.23	>1.22
	5K	148	0.23	30	0.38	<38	0.067	<7
15	5L	150	0.061	5.6	0.024	3.4	0.063	6.5
	7B	160	0.01	7.0	0.018	13.5	0.004	16.5
	8	163	0.032	30	0.011	13.5	0.039	87
	9A	167	0.097	9.6	0.094	13.2	0.028	11.6

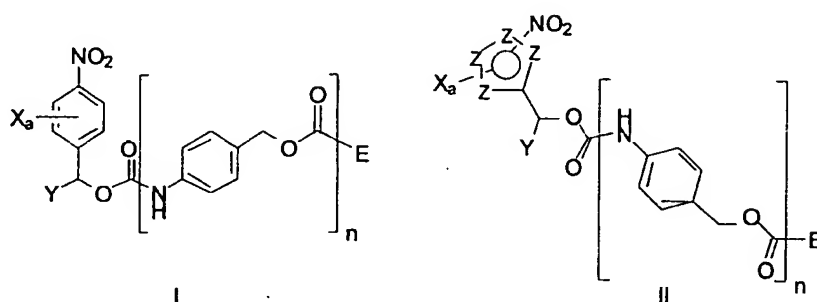
- 117 -

CLAIMS

1. A compound of formula (I) or (II):

wherein:

5 X represents H, C₁₋₆ alkyl or C₁₋₆ alkoxy, said alkyl or alkoxy being optionally



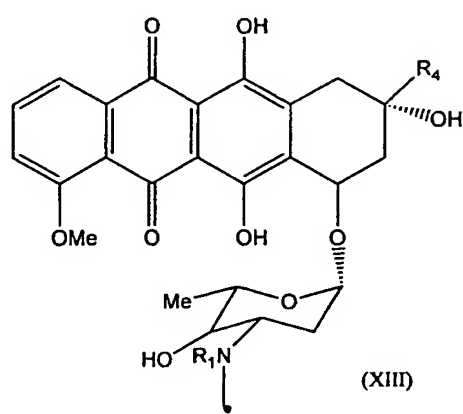
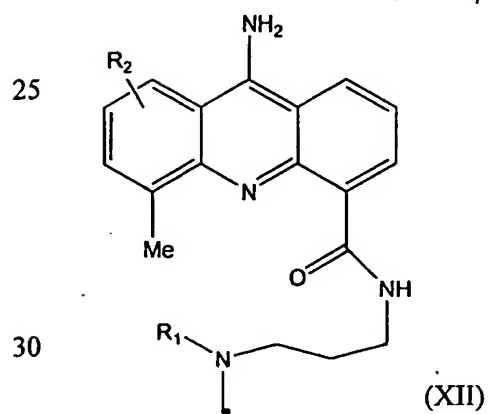
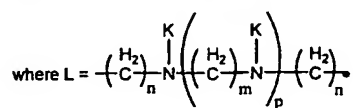
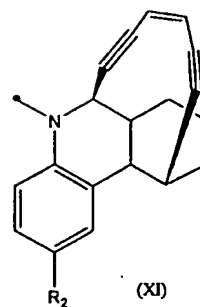
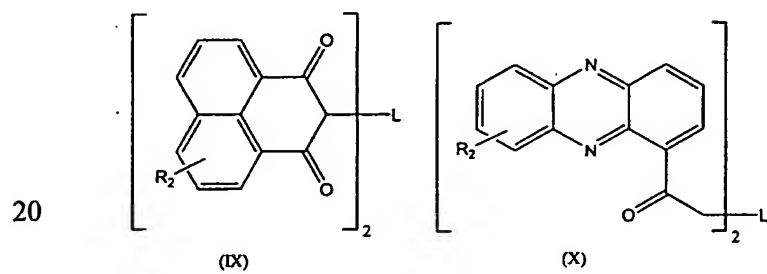
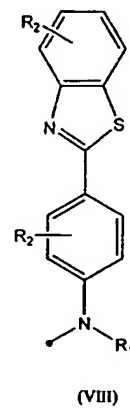
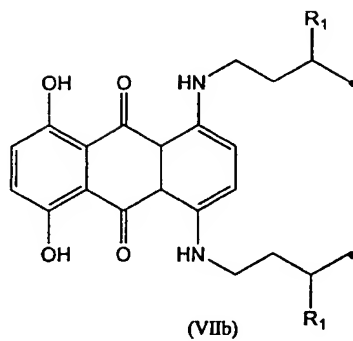
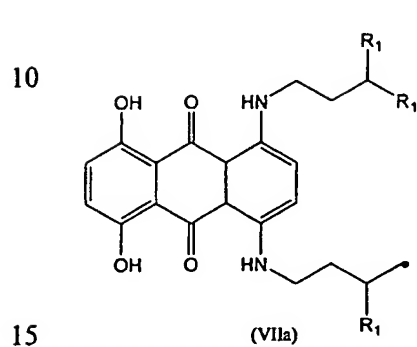
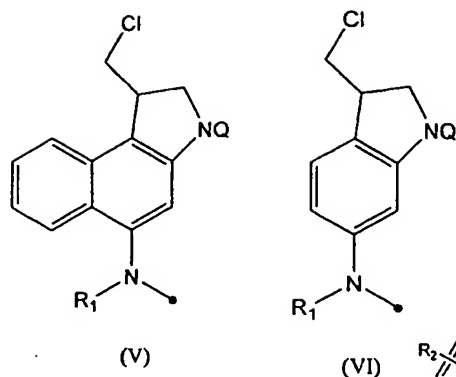
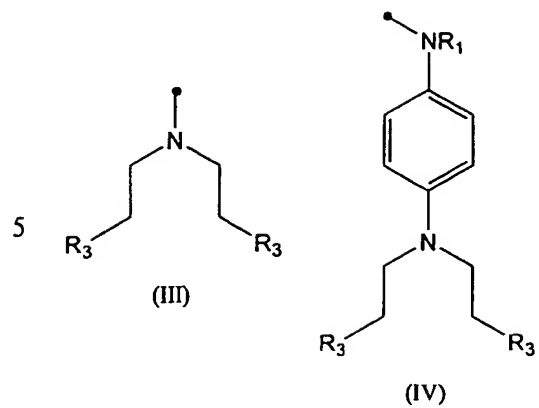
substituted with one or more of the following groups: hydroxy (OH), ether (OR_x), amino (NH₂), mono-substituted amino (NR_xH), di-substituted amino (NR_x¹R_x²), cyclic C₁₋₅ alkylamino, imidazolyl, C₁₋₆ alkylpiperazinyl, morpholino, thiol (SH), thioether (SR_x), tetrazole, carboxy (COOH), carboxylate (COOR_x), sulphony (S(=O)₂OH), sulphonate (S(=O)₂OR_x), sulphonyl (S(=O)₂R_x), sulphoxy (S(=O)OH), sulphinate (S(=O)OR_x), sulphanyl (S(=O)R_x), phosphonooxy (OP(=O)(OH)₂) and phosphate (OP(=O)(OR_x)₂), where R_x, R_x¹ and R_x² are selected from a C₁₋₆ alkyl group, a C₃₋₂₀ heterocyclyl group or a C₅₋₂₀ aryl group; a is 0,1,2,3 or 4; Y represents H or C₁₋₆ alkyl; 1, 2 or 3 of the members Z of the 5-membered aromatic ring are independently selected from -O-, -S-, -N= or -NR-,

15 where R is H or C₁₋₆ alkyl optionally substituted with one or more of the following groups: hydroxy (OH), ether (OR_R), amino (NH₂), mono-substituted amino (NR_RH), di-substituted amino (NR_R¹R_R²), cyclic C₁₋₅ alkylamino, imidazolyl, alkylpiperazinyl, morpholino, thiol (SH), thioether (SR_R), tetrazole, carboxy (COOH), carboxylate (COOR_R), sulphony (S(=O)₂OH), sulphonate (S(=O)₂OR_R), sulphonyl (S(=O)₂R_R), sulphoxy (S(=O)OH),

20 sulphinate (S(=O)OR_R), sulphanyl (S(=O)R_R), phosphonooxy (OP(=O)(OH)₂) and phosphate (OP(=O)(OR_R)₂), where R_R, R_R¹ and R_R² are selected from a C₁₋₆ alkyl group, a C₃₋₂₀ heterocyclyl group or a C₅₋₂₀ aryl group, the other ring atoms being C; n is 0 or 1; and E represents a moiety such that EH is an amine; provided that in formula (I) if a = 0 then Y ≠ H.

2. A compound according to claim 1 wherein EH is a cytotoxic amine.

3. A compound according to claim 2, wherein E is selected from formulae (III-XIII); wherein, R_1 represents H or C_{1-6} alkyl, being optionally substituted with one or more of the following groups: one or more of the following groups: hydroxy (OH), ether (OR_E), amino (NH_2), mono-substituted amino (NR_EH), di-substituted amino ($NR_E^1R_E^2$), cyclic C_{1-5} alkylamino, imidazolyl, C_{1-6} alkylpiperazinyl, morpholino, thiol (SH), thioether (SR_E), tetrazole, carboxy (COOH), carboxylate ($COOR_E$), sulphonyl ($S(=O)_2OH$), sulphonate ($S(=O)_2OR_E$), sulphonyl ($S(=O)_2R_E$), sulphoxy ($S(=O)OH$), sulphinate ($S(=O)OR_E$), sulphanyl ($S(=O)R_E$), phosphonoxy ($OP(=O)(OH)_2$) and phosphate ($OP(=O)(OR_E)_2$), where R_E , R_E^1 and R_E^2 are selected from a C_{1-6} alkyl group, a C_{3-20} heterocyclyl group or a C_{5-20} aryl group; R_2 represents H, C_{1-6} alkyl, C_{1-6} alkoxy, OH, halogen, NO_2 , NH_2 , $NHMe$, NMe_2 , SO_2Me , CF_3 , CN , $CONH_2$ or $CONHMe$; each R_3 is independently selected from Cl, Br, I and OMS; and R_4 is selected from $-C(=O)Me$ and $-C(=O)CH_2OH$; Q represents substituted indole, substituted benzofuran or substituted cinnamoyl; in (IX) and (X), each n is independently from 2-4, and each m is independently from 2-4, and p = 0 or 1.



- 120 -

4. A compound according to any one of the preceding claims, wherein R_x , R_x^1 , R_x^2 , R_R , R_R^1 and R_R^2 are independently C_{1-6} alkyl groups.
5. A compound according to any one of the preceding claims, wherein the compound is of formula (I) and a is 1.
6. A compound according to claim 5, wherein X represents optionally substituted C_{1-6} alkoxy.
- 10 7. A compound according to claim 6, wherein the optionally substituted C_{1-6} alkoxy is in the 2 position.
8. A compound according to claims 7 or 8, wherein the C_{1-6} alkoxy group is selected from methyl, ethyl and n-propyl.
- 15 9. A compound according to any one of claims 6 to 8, wherein the C_{1-6} alkoxy group substituents are selected from hydroxy, methoxy, phosphonoxy, NMe_2 , Nmorph, OCO_2 -tBu, and OCO_2H .
- 20 10. A compound according to claim 9, wherein the C_{1-6} alkoxy group is ethoxy or n-propyl group with a single substituent.
11. A compound according to claim 10, wherein the ethyl or n-propyl substituent is hydroxy.
- 25 12. A compound according to any one of claims 6 to 8, wherein the C_{1-6} alkoxy group is unsubstituted methyl.
13. A compound according to any one of claims 5 to 12, wherein Y is either H or Me.
- 30 14. A compound according to anyone of claims 5 to 13, wherein E is of formula V.

- 121 -

15. A compound according to any one of claims 5 to 13, wherein E is of formula **XIII**.
16. A compound according to claim 15, wherein $n = 1$.
- 5 17. A compound according to any one of claims 1 to 4, wherein the compound is of formula (II) with two Z, one Z being -N= and the other Z being -NR-.
18. A compound according to claim 17, wherein R is either Me or Et.
- 10 19. A compound according to claim 18, wherein R is Et substituted with hydroxy.
20. A compound according to any one of claims 17 to 19, wherein a is 0.
21. A compound according to any one of claims 17 to 20, wherein the -N= and -NR- are
15 not adjacent in the heterocyclic ring.
22. A compound according to any one of claims 17 to 21, wherein E is of formula V.
23. A compound according to any one of claims 17 to 21, wherein E is of formula **XIII**.
- 20 24. A compound according to claim 23, wherein $n = 1$.
25. A compound according to any one of claims 1 to 4, wherein the compound is of formula (II), a is 0, and Z is selected from O or S.
- 25 26. A compound according to any one of claims 1 to 4, wherein the compound is of formula (II), Z is NR, and a is either 0 or 1.
27. A compound according to claim 26, wherein Z is NMe.
- 30 28. A compound according to either claim 26 or 27, wherein a is 1 and X is CO₂Et.

29. A compound according to any one of the preceding claims for pharmaceutical use.

30. A pharmaceutical composition comprising a compound according to any one of claims 1 to 28 and a pharmaceutically acceptable carrier or diluent.

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31. A two component system for the treatment of neoplastic disease which comprises:

(i) a vector encoding and capable of expressing a nitroreductase enzyme in a tumour cell; and

(ii) a compound as defined in any one of claims 1 to 28.

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32. A two component system for the treatment of neoplastic disease which comprises:

(i) a tumour directed antibody linked to a nitroreductase enzyme; and

(ii) a compound as defined in any one of claims 1 to 28.

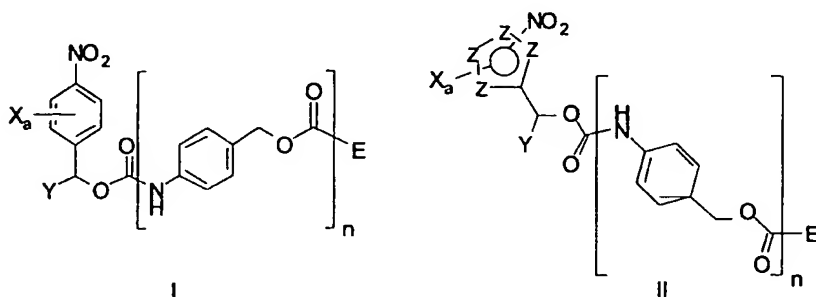
15 33. A compound according to any one of claims 1 to 28, a composition according to claim 26, or a system according to claims 31 or 32 for use in a method of medical treatment.

34. A method of treating neoplastic disease which comprises administering to a patient in need of treatment an effective amount of a compound according to any one of claims 1 to 28,

20 a composition according to claim 30, or a system according to claims 31 or 32.

35. The use of a compound according to any one of claims 1 to 28 for the manufacture of a composition for use in the treatment of a hyper-proliferative disease.

25 36. A method of providing an amine with a protecting group comprising:



(i) providing a plurality of different compounds selected from compounds of formulae (I) and (II)

wherein:

- X represents H, C₁₋₆ alkyl or C₁₋₆ alkoxy, said alkyl or alkoxy being optionally substituted with one or more of the following groups: hydroxy (OH), ether (OR_x), amino (NH₂), mono-substituted amino (NR_xH), di-substituted amino (NR_x¹R_x²), cyclic C₁₋₅ alkylamino, imidazolyl, C₁₋₆ alkylpiperazinyl, morpholino, thiol (SH), thioether (SR_x), tetrazole, carboxy (COOH), carboxylate (COOR_x), sulphony (S(=O)₂OH), sulphonate (S(=O)₂OR_x), sulphonyl (S(=O)₂R_x), sulphoxy (S(=O)OH), sulphinate (S(=O)OR_x), sulphinyl (S(=O)R_x), phosphonooxy (OP(=O)(OH)₂) and phosphate (OP(=O)(OR_x)₂), where R_x, R_x¹ and R_x² are selected from a C₁₋₆ alkyl group, a C₃₋₂₀ heterocyclyl group or a C₅₋₂₀ aryl group; a is 0, 1, 2, 3 or 4; Y represents H or C₁₋₆ alkyl; 1, 2 or 3 of the members Z of the 5-membered aromatic ring are independently selected from -O-, -S-, -N= or -NR-, where R is H or C₁₋₆ alkyl optionally substituted with one or more of the following groups: hydroxy (OH), ether (OR_R), amino (NH₂), mono-substituted amino (NR_RH), di-substituted amino (NR_R¹R_R²), cyclic C₁₋₅ alkylamino, imidazolyl, alkylpiperazinyl, morpholino, thiol (SH), thioether (SR_R), tetrazole, carboxy (COOH), carboxylate (COOR_R), sulphony (S(=O)₂OH), sulphonate (S(=O)₂OR_R), sulphonyl (S(=O)₂R_R), sulphoxy (S(=O)OH), sulphinate (S(=O)OR_R), sulphinyl (S(=O)R_R), phosphonooxy (OP(=O)(OH)₂) and phosphate (OP(=O)(OR_R)₂), where R_R, R_R¹ and R_R² are selected from a C₁₋₆ alkyl group, a C₃₋₂₀ heterocyclyl group or a C₅₋₂₀ aryl group, the other ring atoms being C; n is 0 or 1; and E represents a moiety such that EH is an amine;
- (ii) measuring the rates of fragmentation of the compounds to release EH when the nitro group is reduced and selecting a compound having a desired rate of decomposition; and
- (iii) providing the amine to be protected with a protecting group corresponding to that in the selected compound.

INTERNATIONAL SEARCH REPORT

Int. l. Application No
PCT/GB 00/01612

A. CLASSIFICATION OF SUBJECT MATTER IPC 7 C07C271/28 C07C271/24 C07C271/20 C07D219/04 C07D219/08 C07D209/60 C07D233/91 C07D233/92 C07D277/66 C07D307/71 C07D333/44 C07D231/16 A61K31/27 A61K31/435 A61K31/40					
According to International Patent Classification (IPC) or to both national classification and IPC					
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) IPC 7 C07C C07D A61K					
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched					
Electronic data base consulted during the international search (name of data base and, where practical, search terms used) EPO-Internal, WPI Data, PAJ, BEILSTEIN Data, CHEM ABS Data					
C. DOCUMENTS CONSIDERED TO BE RELEVANT					
Category *	Citation of document, with indication, where appropriate, of the relevant passages				Relevant to claim No.
X	JANE M BERRY ET AL: "5-Nitrofuran-2-ylmethyl group as a potential bioreductively activated pro-drug system" JOURNAL OF THE CHEMICAL SOCIETY, PERKIN TRANSACTIONS 1., 1997, pages 1147-1151, XP002143607 CHEMICAL SOCIETY, LETCHWORTH., GB ISSN: 0300-922X cited in the application page 1147, column 1, lines 23-42 page 1150, scheme 5 and 6 page 1147, scheme 1 <div style="text-align: center; margin-top: 10px;">--- -/--</div>				1,2
<input checked="" type="checkbox"/> Further documents are listed in the continuation of box C. <input checked="" type="checkbox"/> Patent family members are listed in annex.					
<div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> <p>* Special categories of cited documents :</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> </div> <div style="width: 45%;"> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"&" document member of the same patent family</p> </div> </div>					
Date of the actual completion of the international search <div style="text-align: center; font-weight: bold;">27 July 2000</div>			Date of mailing of the international search report <div style="text-align: center; font-weight: bold;">18/08/2000</div>		
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016			Authorized officer <div style="text-align: center; font-weight: bold;">O'Sullivan, P</div>		

INTERNATIONAL SEARCH REPORT

Int'l. Application No.

PCT/GB 00/01612

A. CLASSIFICATION OF SUBJECT MATTER
 IPC 7 A61K31/345 A61K31/415

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>M TERCEL ET AL: "A novel nitro-substituted seco-CI: Application as a reductively activated ADEPT prodrug" BIOORGANIC & MEDICINAL CHEMISTRY LETTERS, vol. 6, no. 22, 1996, pages 2741-2744, XP004135887 OXFORD, GB ISSN: 0960-894X page 1, line 1 - line 13 page 2742, scheme 1, structure 1 table</p> <p style="text-align: center;">--- -/--</p>	1-3, 29, 30, 33-35



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
 "E" earlier document but published on or after the international filing date
 "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
 "O" document referring to an oral disclosure, use, exhibition or other means
 "P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

27 July 2000

Date of mailing of the international search report

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
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Authorized officer

O'Sullivan, P

INTERNATIONAL SEARCH REPORT

Int. Application No.

PCT/GB 00/01612

C. (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>ANTHONY B MAUGER ET AL: "Self-immolative prodrugs: Candidates for antibody-directed enzyme prodrug therapy in conjunction with a nitroreductase enzyme"</p> <p>JOURNAL OF MEDICINAL CHEMISTRY., vol. 37, no. 21, 1994, pages 3452-3458, XP002143609</p> <p>AMERICAN CHEMICAL SOCIETY. WASHINGTON., US</p> <p>ISSN: 0022-2623</p> <p>cited in the application</p> <p>page 3452, column 2, line 10 -page 2, column 1, line 9</p> <p>page 3453, figure 1, structures 1,2,4</p> <p>page 3455, figure 6</p> <p>---</p>	1-3, 29-35
X	<p>WO 98 25898 A (DENNY WILLIAM ALEXANDER ;MILBANK JARED (NZ); TERCEL MOANA (NZ); AT) 18 June 1998 (1998-06-18)</p> <p>abstract</p> <p>figure 1, structure 8a</p> <p>---</p>	1-3, 29-35
X	<p>US 5 633 158 A (ANLEZARK GILLIAN ET AL) 27 May 1997 (1997-05-27)</p> <p>column 4, structure VI</p> <p>column 2, line 61 -column 3, line 24</p> <p>---</p>	1-3, 29-35
X	<p>WO 98 11101 A (DENNY WILLIAM ALEXANDER ;TERCEL MOANA (NZ); ATWELL GRAHAM JOHN (NZ) 19 March 1998 (1998-03-19)</p> <p>abstract</p> <p>figure 4, structure 32d</p> <p>---</p>	1-3, 29-35
X	<p>GB 1 203 960 A (MERCK AND CO) 3 September 1970 (1970-09-03)</p> <p>column 2, line 40</p> <p>---</p>	1
X	<p>GB 1 183 990 A (MERCK AND CO) 11 March 1970 (1970-03-11)</p> <p>page 5, line 33</p> <p>---</p>	1
A	<p>CHEMICAL ABSTRACTS, vol. 108, no. 23, 6 June 1988 (1988-06-06)</p> <p>Columbus, Ohio, US;</p> <p>abstract no. 200788y,</p> <p>GREGORY L KEDDERIS ET AL: "Mechanism of reductive activation of a 5-nitroimidazole by flavoproteins: model studies with dithionite."</p> <p>page 309; column 2;</p> <p>XP002143611</p> <p>abstract</p> <p>---</p>	1-36
A	<p>& ARCHIVES OF BIOCHEMISTRY AND BIOPHYSICS, vol. 262, no. 1, 1988, pages 40-48, NEW YORK, US, US</p> <p>ISSN: 0003-9861</p> <p>---</p> <p style="text-align: center;">-/--</p>	1-36

INTERNATIONAL SEARCH REPORT

Int'l Application No

PCT/GB 00/01612

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	CHEMICAL ABSTRACTS, vol. 127, no. 5, 4 August 1997 (1997-08-04) Columbus, Ohio, US; abstract no. 60258r, MOSES LEE ET AL: "Synthesis of an aminopropyl analog of the experimental anticancer drug tallimustine, and activation of its 4-nitrobenzylcarbamoyl prodrug by nitroreductase and NADH." page 25; column 2; XP002143612 abstract	1-36
A	& BIOORGANIC & MEDICINAL CHEMISTRY LETTERS, vol. 7, no. 8, 1997, pages 1065-1070, OXFORD, GB ISSN: 0960-894X	1-36
A	SCOTT D TAYLOR ET AL: "A general method for the synthesis of O-alkyl N,O'- arylphosphoramidates and its application to the synthesis of a transition state analogue for carbamate hydrolysis" TETRAHEDRON., vol. 54, 1998, pages 4223-4242, XP004113294 ELSEVIER SCIENCE PUBLISHERS, AMSTERDAM., NL ISSN: 0040-4020 page 4224, scheme 1	1-36

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Present claims 1-36 relate to an extremely large number of possible compounds. Support within the meaning of Article 6 PCT and disclosure within the meaning of Article 5 PCT is to be found, however, for only a very small proportion of the compounds claimed. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Consequently, the search has been carried out for those parts of the claims which appear to be supported and disclosed, namely those parts relating to the compounds in which EH refers not to the protecting-functionality in claim 1 (i.e. EH could be simply be RNH_2 where R is the protecting groups drawn and described in claim 1) but to a moiety recognised as an amine-containing drug molecule, present in addition to the LHS protecting moiety, as appears to be desired from the description (i.e. RE, where R is the protecting group, E is the active drug). The search was carried out only in the context of known cytotoxic amines protected as prodrugs using the protecting group moieties described in claim 1.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/GB 00/01612

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9825898 A	18-06-1998	AU 5419298 A EP 0944596 A	03-07-1998 29-09-1999
US 5633158 A	27-05-1997	AU 681337 B AU 2776992 A AU 3521597 A CA 2122036 A EP 0540263 A EP 0638123 A WO 9308288 A JP 7501692 T US 5977065 A US 5780585 A	28-08-1997 21-05-1993 19-02-1998 29-04-1993 05-05-1993 15-02-1995 29-04-1993 23-02-1995 02-11-1999 14-07-1998
WO 9811101 A	19-03-1998	AU 721037 B AU 4403997 A EP 0938474 A	22-06-2000 02-04-1998 01-09-1999
GB 1203960 A	03-09-1970	CH 501645 A FR 1588783 A NL 6717044 A US 3450710 A	15-01-1971 16-03-1970 08-07-1968 17-06-1969
GB 1183990 A	11-03-1970	CH 492717 A FR 1588785 A NL 6717045 A	30-06-1970 16-03-1970 08-07-1968